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CONTEMPORANEOUS ORGANIC DIFFERENTIATION IN THE SPECIES OF PARTULA LIVING IN MOOREA, SOCIETY ISLANDS

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INTRODUCTION

THE present communication records some of the results obtained in the course of an intensive investigation of certain land-gasteropods that live in Moorea. It has been found that many species of the animals in question give trustworthy evidence to the effect that the processes of varietal differentiation have gone forward in recent decades and are still continuing with truly remarkable rapidity. While the degree to which real diversities have been brought about does not amount to that of specific distinction, yet the differences actually demonstrated are exactly the same in nature as those by which true species are distinguished. Because much time is still required for further field-work and for the necessary biometric analysis of the material now in hand—amounting as this does to more than forty-five thousand specimens—it is deemed wise to offer now a general statement of the outstanding qualitative results which will indicate the character of the investigation and the nature of its present conclusions.

Since 1906 the writer has been engaged in a study of the variation, distribution and evolutionary relationships of the species belonging to the genus Partula, which occur in islands of the Pacific Ocean under conditions that are exceptionally favorable for such investigations, for by virtue of their insular situations, their degrees of geographical isolation or proximity are defined with absolute clearness. In general, each group of islands bears species which occur in no other group; there is one known exception which only serves to emphasize the observed rule. Within the confines of a given group the several islands bear their own distinctive forms, again with rare exceptions. Finally, the several valleys of one and the same island constitute the homes of colonies which, in correlation with their nearer situations, are more alike than are any two associations living in separated islands; yet they differ more or less as regards the particular combination of the characteristic island species or with respect to the particular varieties of such species as are represented in them.

My interest in these snails grew out of a desire to study a series of related forms in their natural settings, in an effort to determine the relative values of congenital and "environmental" factors of organic structure. At first it was planned to employ the methods of biometry and genetics in an investigation of the Achatinellidae of the Hawaiian Islands, which served Gulick for his classic studies; but the advice of Mayor and Cooke turned my attention to the Partulae of the Society Islands instead. Mayor had published a brilliant and suggestive paper (Mem. Mus. Comp. Zool. XXVI, No. 2, 1902) on certain species which he had collected in six valleys of Tahiti, where he found the same conditions existing as among the Hawaiian Achatinellidae; therefore it seemed probable that a more thorough and comprehensive study of the species of Partula from the valleys of the inhabited islands would yield profitable results.

Another circumstance of the greatest importance was that the noted naturalist, Andrew Garrett, has devoted years of his life to the collection and study of shells throughout many regions of the Pacific Ocean and elsewhere. He gave particular attention to the genus Partula, and his writings include an extensive and detailed account of the Society Island forms, whose characters and geographical locations he described with extraordinary thoroughness. (Proc. Acad. Nat. Sci. Phila., Vol. IX, Part 1, 1884.) In addition he supplemented his verbal descriptions by a series of outline maps in which he gave the exact localities and ranges of all the Society Island species and major varieties, as these existed during the years of his explorations, 1861–1888. The maps in question were published by Hartman (Bul. Mus. Comp. Zool., Vol. IX, No. 5, 1881); and while they are inaccurate in certain details of outline and proportion, owing to the lack of suitable charts in Garrett's time, nevertheless they are valuable additions to the lengthy publication cited.

Personal explorations in seven different years have been made in several of the insular groups throughout Oceania, but particular attention has been given to the Society Islands—of which Tahiti is the largest and best-known member—because more than half of the hundred and more species of the genus are inhabitants of this one group. During four field trips from 1906 to 1909 inclusive, the islands of Tahiti, Raiatea and Tahaa were completely explored and their species were collected in satisfactory abundance, while Huahine and Borabora were partly worked over. Moorea, which lies about twenty miles to the west northwest of Tahiti, was only partially surveyed in 1907 and 1909, and a return journey was undertaken in 1919 for the purpose of completing the field work of this island. At the later date, however, new collections were obtained in a few of the valleys which were investigated during the earlier years, with the surprising discovery that changes in the constitutional characters of some of the colonies seemed to have taken place even in the few years that had elapsed. Consequently, in the summer of 1923 an almost complete resurvey of Moorea was made, when more than 30,000 specimens were collected from over 60 localities; when this wealth of material was closely examined and compared with the former collections, it afforded ample evidence that varietal differentiation had actually taken place in numerous ways and among several species, not only during the longer interval from Garrett's time to my earliest work, but also in the course of the short period that had passed since 1909.

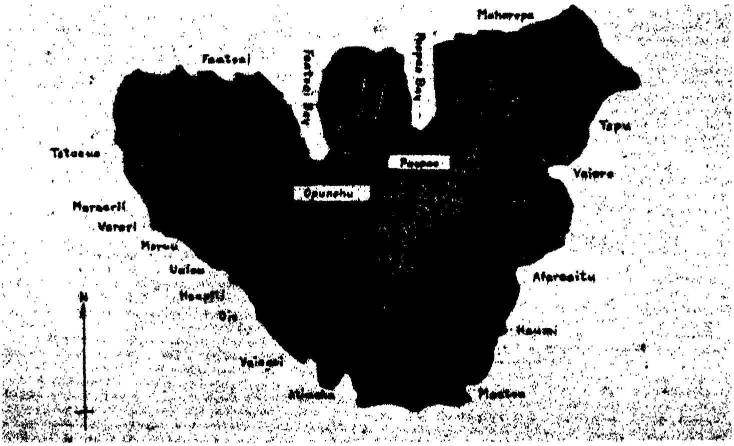
The results of the researches in Tahiti have been published (Carnegie Inst. Pub. No. 228, 1917), and a volume on the species of the Mariana Islands is now in press. The first publication is fundamental in more senses than one in its statement of the nature and purposes of the whole series of researches upon Partula and in its record of the conclusions which have been drawn from the qualitative and quantitative study of the species dwelling in Tahiti. Only a few of the results need to be restated here, on account of their significance in relation to the discoveries in Moorea. *Partula otaheitana* occurs everywhere in Tahiti in one or more of its primary varietal forms. There have been some areal changes since Garrett's time, but these are less important than the constitutional departures displayed by certain colonies and by occasional individuals in some of the localities. Color types unknown to Garrett have been discovered, as well as mutant "giants" and mutant "dwarfs." Sporadic dextral examples were found among sinistral varieties like *P. otaheitana sinistrosa* and *P. otaheitana sinistralis,* and, conversely, reversed individuals were found in colonies composed solely of dextral snails in Garrett's experience. *Partula clara* also exhibited changes which were even more marked than in the foregoing series. It was "very rare" in Garrett's time, and lived only in a restricted area in the southern sector of the island; by 1909 it had expanded its range so as to cover about four fifths of Tahiti, and concurrently its colonies both in the older and in the newer territory had become so differentiated in the qualities of size, shape and coloration that no less than seven primary varieties were distinguishable. *Partula nodosa* also had changed since its first discovery by Garrett, in geographical range and in constitutional respects. These are but a few of the facts which demonstrate the reality of organic differentiation among the Tahitian species, when their later conditions are compared with what they were when Garrett observed and described them.

MOOREA AND ITS ENDEMIC SPECIES

The geographical situation of Moorea is sufficiently indicated by describing it as a near neighbor of Tahiti. It is nearly an equilateral triangle in outline, with one straight side facing north and trending nearly east and west (Plate 1).

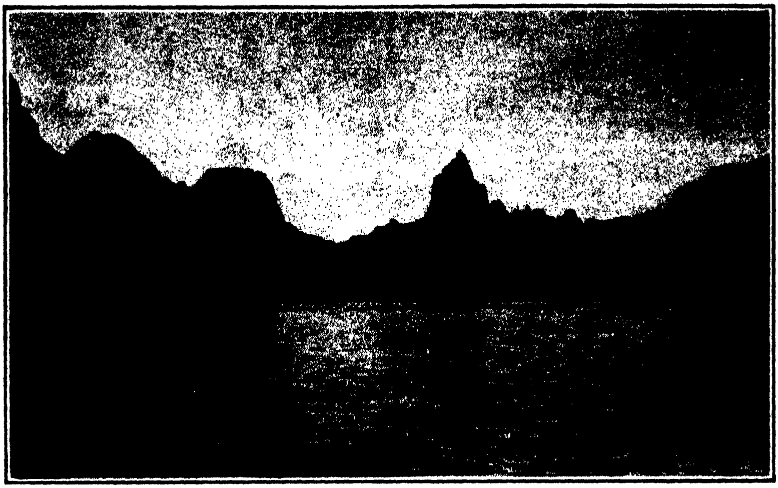
The greatest breadth from east to west is about nine miles, and the axis from north to south is between six and seven miles. The two bays of Faatoai or Opunohu and Paopao indent the northern side; these are clearly interpretable as "drowned valleys," and are features of the

PLATE 1

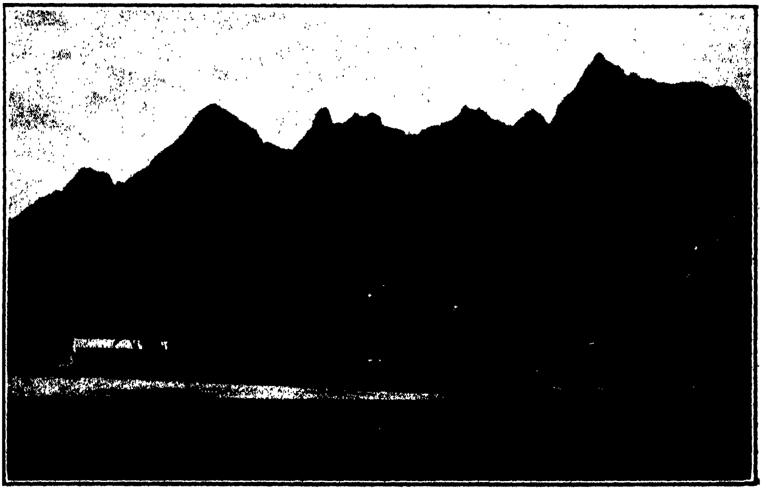


Topography and geography of Moorea, from the official French chart.

PLATE 2

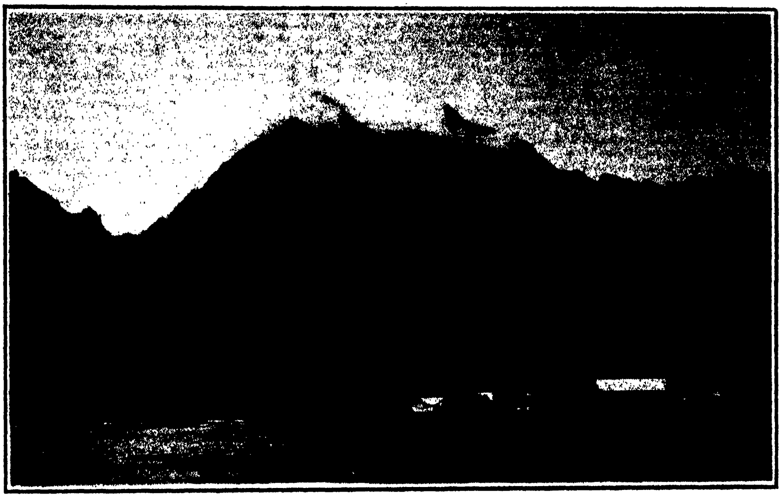


A. Looking south into Opunohu Bay; from left to right (east to west) the mountains in profile are Tohivea, Tamarutofa, Mouaroa and Mouapu.



B. The region of Haapiti, showing a drier coastal barrier between forested valleys.

PLATE 2 (*continued*)



C. The wide valley of Afareaitu, dominated by Mt. Tohivea, over 4,000 feet in height.

island among many which strongly support the view that Moorea was formerly connected with Tahiti and the other islands of the group, from which it has become separated by subsidence. The major structural feature of the island is a huge amphitheater of lofty mountains that were certainly formed as part of a circular wall of an ancient crater (Plates 1 and 2). The highest peaks of this range, from east to west, are Mouaputa, 2,633 feet; Tohivea, 4,040 feet; Tamarutofa, 3,053 feet; Mouaroa, 2,993 feet; Mouapu, 2,547 feet; and Atiati, 2,514 feet. Another prominent portion of the old crater wall is the mass of Mt. Rotui, 2,930 feet high, which stands betwen the two bays on the northern side; this is now disconnected from the main semicircle of high land, excepting by the lower levels of the central valleys named Opunohu and Paopao. The outer slopes of the great mountain chain descend abruptly to about 1,200 feet above the sea where they become more gradual as they trend toward the coast. The descending buttresses are covered with dryer plants toward their outer ends (Plate 2, B), while the valleys between bear the luxuriant vegetation which serves as the dwelling-places of the snails now under discussion. As the lower portions of the dryer ridges constitute barriers to the passage of the animals from one valley to another, such migrations as have been accomplished must have traversed the higher levels where greater moisture and denser vegetation provide more or less suitable avenues. Collections have been obtained in 68 separate localities; some of these are large unitary valleys, such as in Afareaitu (Plate 2 C); others are well-circumscribed areas of lesser size, while still others are subordinate divisions of more open territories, such as the wide amphitheater of Opunohu.

We may now pass to the serial consideration of the species of Partula that live in Moorea. Garrett enumerated four as follows: *Partula lineata* Lesson, *Partula taeniata* Mörch, *P. elongata* Pease and *P. mooreana* Hartman. Pilsbry's list of four agrees in part: *P. suturalis* Pfeiffer instead of *lineata, P. taeniata,* with which *elongata* is combined, *P. mooreana* and *P. erhelii* Mousson. I concur with Pilsbry's view regarding *elongata,* but in my opinion the status of *P. erhelii* is still in doubt. My own work has resulted in the addition of four other species (Nautilus, Vol. XXXVII, 1924), namely, *P. mirabilis, P. dendroica, P. tohiveana* and *P. olympia.* In addition there are certain forms whose position I am not prepared to define without further study, and these may well be passed over in the present review. There are therefore seven species to be taken up: *P. taeniata, P. suturalis, P. mooreana, P. mirabilis, P. dendroica, P. tohiveana* and *P. olympia.* The first of these exists in the greatest numbers and it possesses the widest range, while the others follow in graduated order to the last two, which occur in small numbers in very restricted localities.

PARTULA TAENIATA, MÖRCH.

Within the present limits it is not possible to deal *in extenso* with the numerous subordinate forms which come within the definition of this species. The material is very abundant and diversified, and much time has been devoted to its study in the field and in the laboratory; yet there are several problems concerned with centers of origin, lines of dispersal and even actual relationship which demand more work for their satisfactory solution. However, certain important features of distribution and intrinsic nature are very clear, and some of them fall within the scope of the present discussion.

Like *Partula otaheitana* in its own island, *P. taeniata* now spreads throughout all parts of Moorea, and consequently it is to be regarded as a species of long standing which has been permitted by time and circumstances to multiply and to extend its range widely. Again, like the older species elsewhere, its several colonies have become greatly diversified in numerous characteristics and in various degrees which really require the biometric data for their full demonstration. Sometimes the qualitative differences are plainly evident. In the extreme northwestern colony of Pafatu (Tetaeuo) the shells are large and relatively solid, while in the neighboring valleys from Maraarii to Uufau they are noticeably shorter and stouter. The southern and southwestern associations comprise slender shells which are so thin as to be almost diaphanous; these constitute the primary variety named *elongata*. The northeastern areas are occupied by short, stout and thicker forms, with distinctive features of color as well. The Rotui segment and the Faatoai region bear their own characteristic varieties that are found nowhere else.

While certain color-types have been found which seem to have been unknown to Garrett, and which may perhaps be the product of mutation since his time, no doubt whatever arises with regard to the novelty of *reversed* specimens that have come to light in two widely separated localities. This species has been described as universally dextral, and no shells with the sinistral coil have been collected or noticed heretofore, so far as the literature and museum series are to be trusted. That ample series of specimens have been observed is proved by Garrett's reference to the "prodigious numbers" and "thousands" of individuals which came under his notice. My own collections of *taeniata* comprise more than twenty-five thousand adults and about an equal number of adolescents and advanced embryonic young, all of which are dextral with two new-found exceptions. In 1923, one dark-colored *reversed* adult was taken in a small valley near Maraarii, in the northwestern region; it is a typical *taeniata* of its territory. The animal was not gravid, and therefore it can not be known what kind of young it might have produced. Another sinistral individual of this species was also discovered in 1923 in Atimaha Valley—an area of much importance for the study of *P. suturalis* and *P. mooreana,* at the extreme southern end of the island. This specimen was infertile, like that of the northern valley. Both of the reversed snails were full grown, and obviously their origin by mutation must have occurred from one to two years prior to their discovery.

We have in *P. taeniata* a species that has long been dispersed throughout the habitable territories of Moorea, in contrast with others to be described hereinafter. Yet it can not be said to have reached the end of its evolution, for the processes of differentiation by which its varied representatives have come to be organically distinguishable in separated valleys seem to have continued to some extent during the years that have elapsed since Garrett made his fundamental studies. In my own experience with *taeniata,* the most notable discoveries are the two reversed individuals, which can not be anything but products of recent mutation. It is true that the episodes of sinistral transformation are excessively rare; to establish a true variety with the reversed coil would require the coincident origin of several individuals with the novel character, or the direct transmission of the changed body-form to the progeny of the mutant animal. As the case of *taeniata* now stands, we can not say whether the occurrence of such mutations would be futile, or, if repeated, would lead to the establishment of a new variety or species. Fortunately the data are far more satisfactory in the cases still to be dealt with, in whose terms the history of *taeniata* is in part to be interpreted.

Partula suturalis Pfeiffer

The material facts in the case of *Partula suturalis* constitute the most satisfactory body of proof that organic differentiation is a contemporaneous process and that the production of new diverging forms having the status of varieties is the requisite initial phase in the origin from common parentage of more widely separated types that may justly be denoted species. This case is complete on its own evidential merits, and it is still further strengthened by the demonstration of exactly parallel episodes in the histories of other species living in Moorea.

The description given by Garrett definitely portrays the condition of the species and of its varieties for his time, as regards the intrinsic nature of the various colonies and their geographical distribution. My own observations were made at the earlier period of 1907 and 1909, and at the later period of 1919 and 1923. It is therefore possible to compare my earlier observations with those of Garrett and my own later experience with what I had found in 1907 and 1909. When all the data are assembled, they provide convincing proof that the species has spread to an astonishing degree since Garrett's time, that its various colonies have changed in certain definite genetic qualities, that mutations have arisen in various characters such as shape and size and direction of the coil, and finally that mutations are continuing to arise.

Garrett (*loc. cit.*) deals with this species under the name of *P. lineata* Lesson, but in a letter to Hartman he expressed his regret that he used Lesson's name. There is no question as to the shells which Garrett had in mind, however, and we follow Pilsbry in the use of *P. suturalis* Pfeiffer for this species. Garrett's statements are so important as to demand full and exact quotation, as follows:

> This beautiful arboreal species is found in great profusion in Vaianai valley, on the southwest coast of Moorea, where it occurs in company with *P. Mooreana* and *P. elongata.* It also exists in considerable numbers in a small valley about two miles to the westward, associated with *P. taeniata* and *elongata.*
>
> I first discovered this species in 1861 and obtained several hundred specimens, *all* collected on the eastern side of the stream that flows through the valley of Vaianai. They were *all* dextral, and were so described by Pease, under the name of *vexillum.* On a second visit, in 1875, I took over 2,000 examples, *all* gathered on the *western* side of the stream, and was surprised to find many sinistral forms among them. At the same time I found about a dozen specimens, *all* sinistral, in a large semicircular valley on the opposite side of the island. They were probably stragglers from Vaianai.
>
> It is noteworthy that no reversed *Partulae* were found in any other part of the island except on the western side of the stream in Vaianai, and the above-mentioned stragglers taken on the opposite coast. The same side of the stream is also the home of the sinistral *P. Mooreana.*
>
> Several miles to the eastward of Vaianai, in a large valley named Oahumi, it is found equally as abundant as in the former location. The Oahumi shells, which are slightly modified (= *strigosa* = *alternata*), gradually inosculate with *lineata.* It occurs, also, sparingly in a valley more to the eastward, where it is associated with *P. taeniata* and *striolata.*

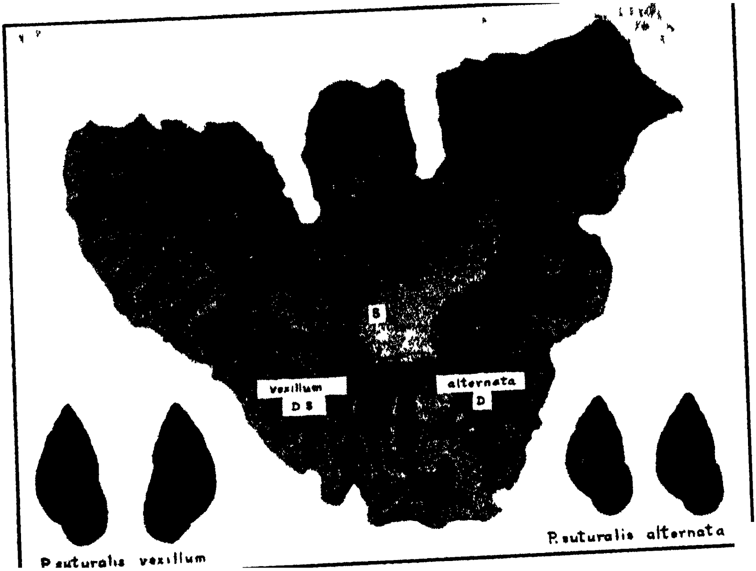
These statements are satisfactorily definite as to the geographical facts and also as regards the associates of *suturalis* in the several localities mentioned. Furthermore, they properly distinguish the two primary divisions of the species, denoted *P. suturalis vexillum* and *P. suturalis alternata* in the present article. The name "Vaianai" is still in use for an area which is really a little *west* of the southernmost point of the island, instead of southeast, as Garrett described it and sketched it in the outline map published by Hartman. The valley to the westward, mentioned in the first paragraph of the quotation, is undoubtedly Uufau, which is exactly two miles from Vaianai. The area "on the opposite side" of the island where Garrett's sinistral stragglers were found is Opunohu. The last paragraph refers to *P. suturalis alternata* as occurring in "Oahumi," which should be Haümi, and in the great valley of the Afareaitu region to the eastward of Haumi, which last is clearly specified as the limit in this direction of the extent of the species in Garrett's experience.

But far more important than the matter of mere geographical extent are the data relating to the nature of the local colonies as regards the modes of coiling of their components. The species was fundamentally dextral in 1875, but in Vaianai sinistral individuals existed to the number of fifty out of two thousand, as Garrett states in a later paragraph. None were noted in the valleys to the westward or to the eastward, and surely Garrett would have mentioned them if they had existed. The "stragglers" of sinistral nature which Garrett found in Opunohu are especially noteworthy for future consideration; and in view of the words used by Garrett, it is fair to conclude that he discovered no dextral examples of *suturalis* accompanying them in the Opunohu region. The condition of *P. suturalis* in 1875 is therefore known with substantial definiteness (Plate 3 A).

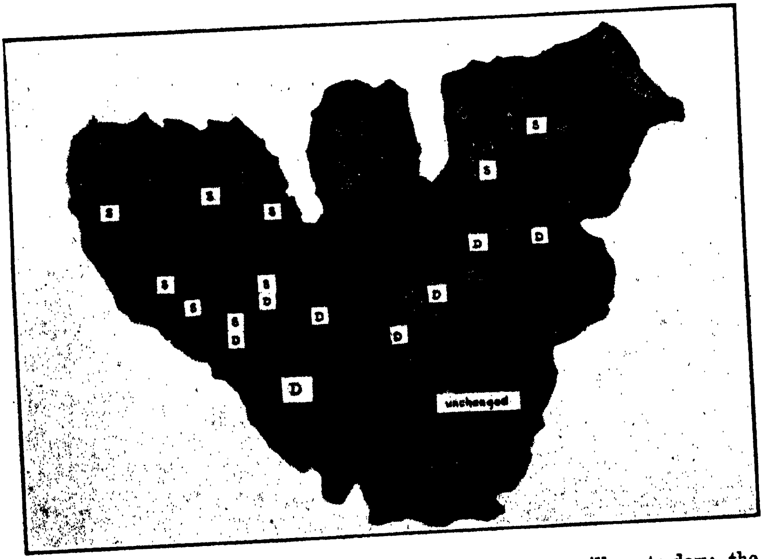
To-day the territorial extent and the intrinsic qualities of the colonies of *suturalis* are vastly different (Plate 3 B). While *P. suturalis alternata* is substantially the same, so far as my present knowledge goes, *P. suturalis vexillum* has spread into almost all of the habitable areas of Moorea, excepting only those of the Rotui sector, which lies between the two great bays of Faatoai and Paopao. And when we pass from the old area of occupation to the new-won territories, the species changes its character from purely *dextral* to exclusively *sinistral* in both directions.

It is beyond the purposes of the present article to deal fully with the evidences so briefly summarized in the foregoing statement, but some of the details are required in order that the nature of the proof may be clear. Beginning with the original valley of Vaianai, we have Garrett's observation of 1875 that two and a half per cent. were sinistral, while in the years of my own work no

PLATE 3



A. The distribution and nature of *Partula suturalis* as described by Garrett.



B. The distribution and nature of *Partula suturalis vexillum* to-day; the arrows show the extent and direction of recent dispersal.

sinistral specimens of *suturalis* have been found in that locality; hence we must conclude that Garrett's reversed specimens were the products of a transitory and sporadic phase of mutation. This conclusion is supported by my own findings in the neighboring valleys of Oio and Haapiti. In the former area, reversed individuals amounting to 1.3 per cent. of the adult population were found in 1919, while in 1923 the sinistral type was absent in the same territory; in Haapiti, about 7.5 per cent. were sinistral in the 1907 collection, but in 1923 not a single snail of this kind was observed.

Passing northwestward, we come to Uufau Valley in which Garrett found no reversed snails at all. In my own collections of 1907, the sinistral component amounted to *87 per cent. of the adult population,* while in the series obtained in 1923 this component had dropped to about 71 per cent. of the adult population and to 68 per cent. of the embryonic series. To the northwest beyond Uufau, where the species did not occur in Garrett's time, the valleys are now occupied by *suturalis* in numbers that diminish as we approach the western limit of Tetaeuo, in such a way as to indicate a recent immigration into this sector of the island. Here the species is uniformly *sinistral* with the exception of very rare mutations to dextrality, such as one adult in Moruu Valley and one embryonic snail in the Maraarii collection.

Taking up the colonies of the great amphitheater of Opunohu across the mountains, we must return to the neighborhood of Vaianai Valley, because that area was the center from which the present Opunohu populations must have been derived. This conclusion is inevitable from Garrett's statements that in 1875 sinistral examples were found in Vaianai and Opunohu; the population of Uufau Valley was dextral at that time. Having established themselves across the mountains in Opunohu, the migrants produced descendants which spread to the west, northwest and north, and also to the east and northeast; but the species was prevented by the lack of suitable avenues of vegetation from crossing the low lands to the median northern sector of Rotui. To-day *suturalis* is *exclusively dextral* in that part of Opunohu which is nearest to the pass from Vaianai, namely, Maramu. To the westward, it is dextral as far as Roroie Valley, near the foot of Mt. Mouapu, but thereafter the colonies are mixed, until with the approach to the Vairahi division of Opunohu Valley the dextral individuals diminish in numbers and appear only as casual mutations among the prevalent sinistral forms, as in Tupuna and Vaiana (not to be confused with Vaianai). Still further north, the species has recrossed the encircling mountains so as to penetrate into Urufara Valley, and, quite independently, into Faatoai Valley, which is the last habitable area in this direction away from the original territory of Vaianai. From my own observations, it is plain that the species is now more abundant in the extreme valleys than it was in the earlier years of 1907 and 1909.

The eastern flood of migration from the first established settlement in Maramu is quite as interesting as the western. All the colonies are now dextral, where the advancing tides of successive generations must first have been sinistral, until we pass Mt. Mouaputa. In the uppermost Paopao region, the species is *sinistral,* and it remains so in the contiguous northern valley of Paraoro, and in the extreme valley of Maharepa; the last-named area has been invaded since 1907. Beyond Mouaputa, *suturalis* has crossed the mountains to the outer circle of valleys of the Vaiare sector, where dextral and sinistral components now exist in company; but in the valleys northeast of Vaiare the former disappear and the colonies comprise only reversed individuals.

In summary, therefore, the most remote colonies at both geographical extremes are found to be alike in their exclusive sinistrality. It seems certain that the sinistral "stragglers" found in Opunohu by Garrett constituted the forefront of a wave of migration which has since spread to the very extremes of the habitable territories.

As they spread more and more widely, the individuals constituting the advance lines retained the sinistral form of coil, but their descendants and their followers returned to the dextral mode as a wave of dextrality swept after. The present lack of reversed snails in Vaianai and Haapiti, where they formerly existed, and the diminished numbers of this kind in 1923 in Uufau, where they constituted 87 per cent. of the population in 1907, are illustrative details among the many which justify such a summary statement of the recent history of *suturalis.*

The biometric analysis of the numerous colonies brings to light other kinds of transformation which are entirely independent of the qualities of spirality. When the standard characters of the shells are defined in terms of their ranges, averages and indices of variation, the several colonies exhibit differences of real significance, which of course have come about as the snails have established their somewhat isolated settlements in places more or less remote from the original territory of Vaianai. Some of the sinistral members of the western Opunohu series are as short as 16 mm, and are therefore no longer than their *taeniata* associates, while some dextral shells on the Opunohu side, not far from the pass leading from Vaianai, attain a length of more than 24 mm. There are also notable diversities in shape as well. Such differences seem to be truly genetic as the colonial statistics indicate. Furthermore, there are types of coloration that are not described by Garrett, and it seems legitimate to interpret these as new mutations from the ancestral modes of fasciation and coloration.

In brief, then, *Partula suturalis* proves to be a species comprising two primary divisions, *vexillum* and *alternata,* which have had very different histories in recent times. The latter has remained substantially the same, hemmed in as it is by relatively impassable barriers of mountain and dry ridge, while the former has changed in several ways and to remarkable degrees during the last few decades. The occupation of larger territory is far less noteworthy than the alterations in organic nature which are exhibited in spirality, size, proportionate measures and in coloration.

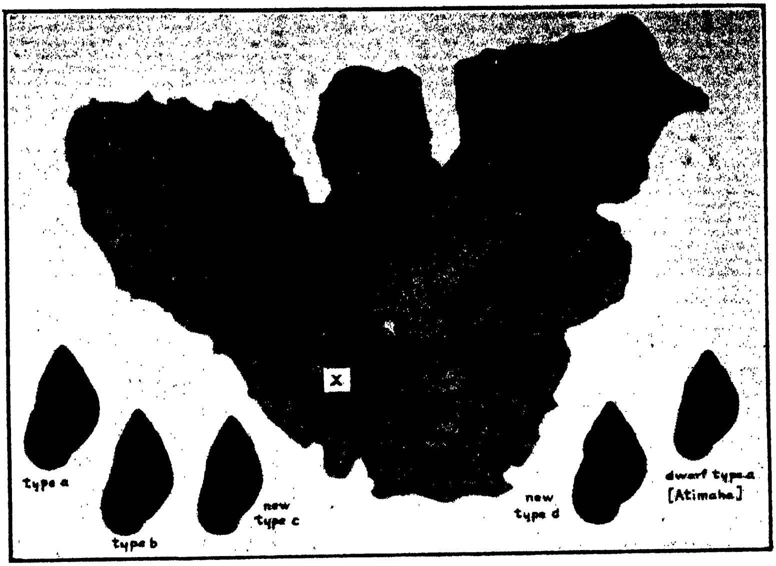
PARTULA MOOREANA, HARTMANN

The history of *P. mooreana* is very much like that of *P. suturalis;* when Garrett explored the island, it existed only in a restricted locality, from which it has since spread, though less widely than *suturalis.* In its original metropolis and in several of the newly occupied regions, it has produced novel mutations of several kinds. It is an interesting coincidence that the former limited habitat of this species was Vaianai Valley, which was also the center from which *P. suturalis vexillum* has now become so widely dispersed.

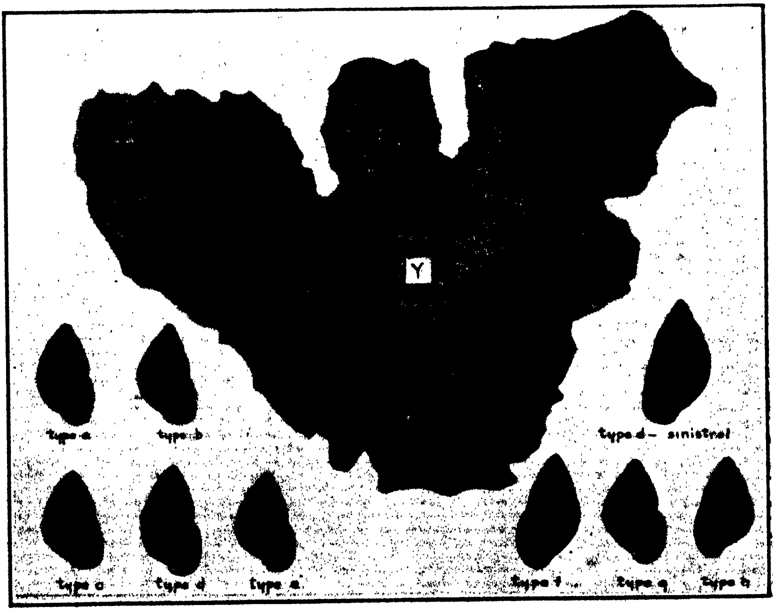
Garrett discovered the first specimens of *P. mooreana* and sent them to Hartmann, who published their description in 1880 (Proc. Acad. Nat. Sci. Phila., 1880, p. 229). In his monograph on the Society Islands species, Garrett gives the following definite statements concerning *mooreana* (pp. 59, 60): "This arboreal species is abundant, and restricted to Vaianai Valley on the southeast coast of Moorea, where it shares the metropolis of *P. vexillum* Pse." "It is *always* sinistral, . . . " "A variety with three narrow pale brown revolving bands is not infrequent." The error in assigning Vaianai to the southeast instead of the southwest coast is a trivial one, already noted (cf. Plate 4 A).

In the course of my own studies, the species was duly found in 1907 in the original locality, from whence large numbers were also taken in subsequent years. In 1909 it was also discovered in the Maramu subdivision of Opunohu, immediately across the mountains from Vaianai; although its relative numbers were few in that year, by 1923 the species had so increased as to constitute nearly 80 per cent. of the Partula fauna in Maramu. Furthermore, it has been taken in valleys on either side of Maramu, its first foothold across the divide from

PLATE 4



A. *Partula mooreana.* X marks the original locality; the arrows show the extent and direction of recent dispersal.



B. *Partula mirabilis.* Y marks the probable original locality.

Vaianai, in areas that extend over a lateral distance of about two miles. Returning to the outer side of the island, the species was found to constitute about 10 per cent. of the Partula population in Atimaha Valley, adjacent to Vaianai on the east, where in 1923 it had increased in relative numbers so as to form almost 80 per cent. Some of the western valleys have also been recently invaded. Oio, next to Vaianai, contained about 15 per cent. of *mooreana* in 1919, and nearly 40 per cent. in 1923; the Haapiti collections of 1907 included none of this species, while those of 1923 comprise about 30 per cent. of *mooreana*. Clearly, therefore, the species began its geographical extension some time after Garrett made his pioneer studies. It spread across the mountains to Maramu and beyond to neighboring areas, and it crossed the eastern boundary of Vaianai to Atimaha at approximately the same time, increasing in relative numbers in both territories; at a somewhat later date it invaded Oio and Haapiti, in which it still exists on the heights from which it has not yet had time to descend to the forested areas of lower altitude.

The changes in the intrinsic nature of the *mooreana* colonies are more significant than the geographical expansion of the species as a whole. Garrett describes only two color-forms in the original Vaianai association, namely, the prevalent unbanded type (Plate 4A, type a) and a form with three pale brown bands (Plate 4A, type b); it is unfortunate that he does not give the exact numerical proportions, but states only that the latter is "infrequent." My own observations make it certain that the banded form has diminished in recent years in Vaianai; whereas the 1907 collection comprised 14 per cent., the series of 1919 and 1923 included respectively only 2.6 and 2.8 per cent. of the fasciated individuals. The same decrease in the relative numbers of the banded type is observed on the Maramu side, where in 1909 nearly 44 per cent. were banded, while in 1919 there were only 8 per cent. of this kind, and less than 2 per cent. in

1923! The Oio, Haapiti and extreme Opunohu colonies furthest from Maramu are composed of the unbanded forms solely. The Atimaha collection comprises about 1.5 per cent. of this three-banded kind.

Other color types have been discovered which are apparently new since Garrett's time. The first is light-colored, with a single narrow median band of brown (Plate 4A, type c). This was *not present* in my own Vaianai collections of 1907 and 1919, but in 1923 three out of 2,000 specimens of all ages were discovered. In Atimaha, 1 per cent. in 1919 and 4 per cent. in 1923 were of this kind. Apparently, therefore, the single banded type is new in point of time, and it seems to be on the increase in Atimaha. Whether the novel coloration has been produced independently in Vaianai and Atimaha or whether it was a product of mutation in Atimaha, whence it was introduced into Vaianai, is a problem which requires additional data for its solution; but the statistical data tend to support the former interpretation.

Another new color type is dark-colored, with a narrow median corneous band, which is therefore the exact converse of the one-banded form (Plate 4A, type d). This was discovered in 1923 in the upper part of Vaianai Valley, *in the same area which had yielded the extensive collections of previous years in which it did not exist.* It occurred in greater numbers than the type with the single stripe.

The next point is one which would not be evident without the biometric analysis of the several colonies; it is the dwarfed size of the shells which form the Atimaha association (Plate 3A, type *a* Atimaha). The general length of the Vaianai individuals is approximately 17.5 mm, while those of Atimaha center about 15.5 mm; thus the former are about one eighth longer than the latter. It would seem that the first migrants from Vaianai into Atimaha were shorter than the average of their kind, and that they transmitted their abbreviated stature to their descendants of to-day. It is not justifiable to refer the reduced size to peculiar environmental conditions obtaining in Atimaha, because no such external factors can be discovered and also because the associated species display no parallel diminution in length, as they should if environmental conditions *per se* were responsible for the dwarfed stature of *mooreana* in this locality. The converse of this case is provided by the extreme western colonies of the Opunohu region, where the shells are statistically larger to a degree that is well beyond probable error. Again there are no external circumstances to which the different quality may be referred, and again there are no parallel enlargements to be observed in the accompanying species.

At the last, we come to the most notable mutation as yet discovered in this species, namely, the production of *dextral* individuals. A single right-handed adult was found in 1923 in Maramu, whose affinities with the prevalent sinistral type were indicated by its patent characters of color, size, shape and coloration, while in addition it bore in its brood-pouch *two sinistral young.* Again, in the 1923 collection from Tefeo Valley, adjacent to Maramu on the east, a sinistral *mooreana* was found which contained *two dextral young.* No clearer proof of sporadic dextral mutation could be obtained. The rarity of such an episode may be judged from the occurrence of only these three individuals with direct coil out of a total array comprising well over 9,000 specimens of *mooreana* of all ages.

The words of the concluding paragraph summarizing the observations on *suturalis* might be repeated, so far as they refer to observed changes in the way of territorial expansion, and varietal differentiation; the phenomena are clearly parallel in the recent histories of *mooreana* and *suturalis.*

PARTULA MIRABILIS CRAMPTON

The species now to be considered is the first among several that have come to light for the first time in the course of the present researches; they have been deemed so noteworthy as to deserve special notice and record (Nautilus, Vol. XXXVII, 1924). In view of Garrett's long and careful studies in the Society Islands, it was very surprising to find novel forms which were unknown in the literature and entirely lacking in the extensive collections made by Garrett, sent by him to Hartmann and others, and now deposited in the museums of Honolulu, Washington, Cambridge, Philadelphia and Pittsburgh. I have seen all these collections and it is certain that the new species are not represented in them; it is especially important that they are not contained in the Hartmann collection at Pittsburgh, as Garrett wrote to Hartmann that he (Hartmann) had received specimens of all the forms discovered in Moorea. There can be no question as to the thoroughness of Garrett's explorations, and we must attribute his failure to find the new types to the peculiar circumstances of their distribution in his time.

Partula mirabilis was discovered in 1907 in the Maramu subdivision of Opunohu Valley, and in subsequent years it has been taken in eleven other more or less contiguous localities. About two thousand specimens are in hand. The circumstances under which they exist, and their own qualities are such as to demonstrate exactly the same kind of history which *P. suturalis* and *P. mooreana* have undergone. Apparently the parent colony was so small in the years of Garrett's work as to escape his attention, thus presenting at that period the same restricted condition which we now find in two species later to be described. Like *suturalis* and *mooreana*, but much later in point of time, *mirabilis* began to extend its area of occupation; concurrently it seems to have passed into a period of active mutation, with the result that in the qualities of coloration its colonies exhibit greater differentiation than an equivalent number of associations of any other Moorean species, in an area of equal size. Although the species is predominately dextral, very exceptional sinistral individuals have been found in one circumscribed colony.

The pertinent facts are illustrated in plate 4B. Apparently the original center was somewhere in Opunohu in the neighborhood of Maramu, Tefeo or Irua, whence the tides of migration spread to the west and to the east, and also northward across the lower ground to the southern valleys of the Rotui sector. The 1923 collections from the last-named region comprise far higher relative numbers of *mirabilis* than did those of 1909, hence the northward migration appears to have been accomplished in recent years. Another episode of territorial enlargement is still more proximate in time, namely, the invasion of Vaianai Valley from Maramu; in 1923 this species was found in the upper and middle portions of Vaianai, *from which it was entirely absent* as recently as 1919, when my own collections of all species amounted to thousands of specimens.

The qualitative facts of colonial differentiation may be briefly set forth, without entering into the quantitative details, although the latter are additionally significant on their own merits. The species as a whole comprises at least eight color-types, all of which are shown in Plate 4B: (a) light and uniform in general, with darkened apex; (b) boldly strigated transversely; (c) medium in color with reddish spire; (d) deep brown; (e) with three revolving bands; (f) with a broad revolving zone of deeper color; (g) dark, with a narrow longitudinal band to the left of the median line; (h) dark, with a narrow median corneous band. These are entitled to be called genetic types, inasmuch as they are repeated in offspring, along with young of other kinds whose coincident presence proves that the several color-classes interbreed freely. Each one of the localized associations possesses its own characteristic color composition, marked by the presence of only some of the whole array of color-types and also by particular numerical relations of the component color-classes, which proportions are not duplicated elsewhere. Using the symbol letters for the different kinds, the Maramu colony comprises *c.d.g.* and *f.* types, stated in their order of numerical abundance, while the association in Faataofe, a small valley to the west, is made up of *d.e.h.* and *a.* individuals. East of Maramu, the *c.e.d.f.* and *g.* types are present. On the southward slopes of Rotui the colonies consist of *c.b.f.* and *g.* individuals only. Finally, the recent migrants into Vaianai are *d.f.* and *e.* forms. From these facts, it may be concluded that the *h.* type is a local mutation in the Faataofe locality, and that the *b.* type has come into existence more to the eastward of the original home of the species. Regarding the *e.* form, it may be that this distinctive pattern has independently arisen in the Faataofe, Irua and Vaianai areas, but it is conceivable that it existed earlier in the central Maramu association, from which it spread into the surrounding valleys where it now persists, while it has disappeared in the locality where it formerly occurred. But whatever may have been the modes by which the various types have originated, the result at the present time is a condition of colonial diversification, entirely similar in principle to what is displayed by *suturalis* and *mooreana*, but greater in degree when the smaller area is taken into account.

Finally, the important observation is recorded that in one of the newly established colonies of *mirabilis* sinistral mutation has occurred. The area is Faataofe Valley, in which three reversed adults were found (Plate 4B, type d sinistral); they belonged to the prevalent color-class of dark brown shells. In each of two, a single *dextral* young snail was found, while the third reversed individual contained two *sinistral* young, which therefore perpetuated the transformed character of their parent.

The case of *mirabilis* is very interesting both on its own and on its comparative merits. My personal observations prove its territorial expansion within recent years, and Garrett's failure to record it at all signifies an unusually small area of its occurrence in his time. The history of the species accords with those of *suturalis* and *mooreana* in all essential respects, even to a correspondence in contemporaneous production of novel mutant individuals differing from the generality in the direction of the spiral coil.

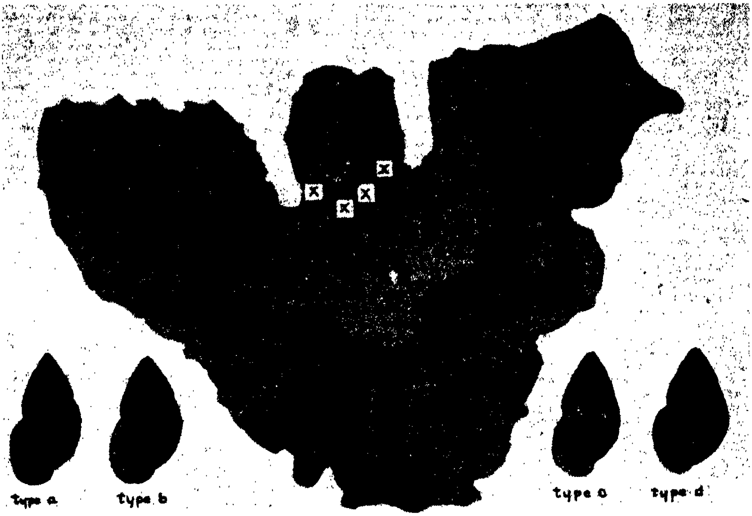
PARTULA DENDROICA CRAMPTON

The second of the newly found forms, *Partula dendroica,* occupies a much smaller territory than any of the foregoing; it therefore presents a condition like that of *mirabilis* not long ago, and of *mooreana* and *suturalis* in more remote decades. It was first discovered in 1909 in Vaihiaiia Valley, on the southwestern side of the Rotui sector (Plate 5A), and in subsequent years the species was taken also in valleys on the southern and southeastern aspects of the same mountain mass. The western, southern and eastern slopes of Mt. Rotui are more abrupt than those of the northern seaward side, and their valleys differ correspondingly; thick vegetation grows to an altitude of more than 1,100 feet in the gullies of the southern face, but the heights to which the dense vegetation extends gradually diminish on both sides toward the north. *Partula dendroica* has been found in seven of these subsidiary areas, where it lives upon the trees and shrubs of larger size. The animals are exclusively sinistral.

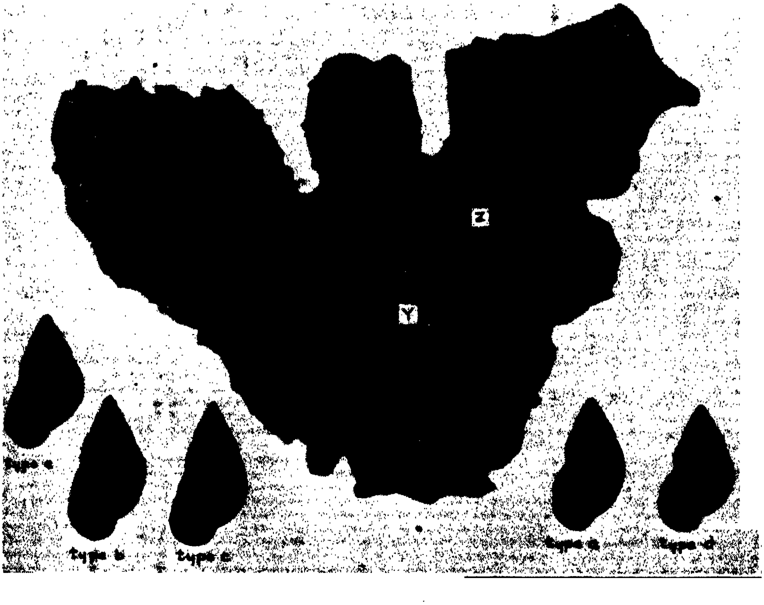
Four color-types are presented as follows (Plate 5A): (a) devoid of bands and with apical darkening; (b) with one median longitudinal band; (c) with four revolving bands; (d) with a broad median zone of dark color. The colonies to the west are composed of types *a.b.* and *c.,* stated in the order of their numerical frequency; the southern colonies differ, sometimes comprising the *d.a.c.* classes and sometimes with the composition *d.c.a.b.* At the southeastern extremity of the whole range only the *a.b.* types are present.

The distribution of *dendroica* suggests that it has recently spread from a more restricted territory which, like that of *mirabilis* some time ago, must have been overlooked by Garrett. The western and eastern borders of

PLATE 5



A. *Partula dendroica,* living in the localities marked X.



B. *Partula tohiveana,* living at the place marked Y.

C. *Partula olympia,* occurring in the region marked Z.

the area now occupied are not marked by barriers to the further extension of the species, whence it would appear that these outer regions have not long been occupied. While there is some degree of intrinsic differentiation of the several valley associations, the differences are far less in degree than those displayed by the *mirabilis* colonies; such considerations also indicate that the peripheral associations have not long been separated from a central parent colony, in which all the principal color-types may be presumed to have been present.

Thus the case of *dendroica* stands next to that of *mirabilis,* as regards the smaller area of occupation, its more recent dispersal and its lesser degree of internal differentiation.

PARTULA TOHIVEANA CRAMPTON

Like the foregoing, *P. tohiveana* is a newly discovered species whose existence in Moorea was entirely unexpected. It was found in 1923 on the high inner slopes of Mt. Tohivea, the dominating mountain of the island (Plate 5B). Several hundred individuals were taken in an area not more than four hundred yards in lateral extent at an altitude of 700 to 900 feet above sea-level. All the animals collected were sinistral.

Despite the small size of the colony, the shells are definitely differentiated in color characteristics (Plate 5B); the three types are (a) plain, (b) encircled with two or sometimes four vaguely-defined bands, and (c) with a broad median zone of color. The distinctions in color pattern are hereditary. The composite nature of the association is noteworthy, for it is here clearly evident that diverse genetic types of color can arise in one and the same compact area of habitation.

This species is significant in certain other connections. It is distinguished from the other sinistral species of Moorea by its general make-up, and by special features such as the roundly dentated lip and simple conical spire. But these very distinctions mark it as a relative of a group of forms living in Raiatea, an island situated about 120 miles to the west northwest. The latter group comprises *P. callifera, P. dentifera* and *P. formosa,* all of which are dextral. If the common characters in question really do signify a community of origin of *tohiveana* and the Raiatea species, then there must have been a prior land-connection between Moorea and Raiatea; upon this larger land-mass the ancestors must have been dextral and sinistral, exactly as *P. otaheitana* and *P. suturalis* now are. With the subsidence of the connecting bridge, the colonies of Moorea and of Raiatea were isolated. Those of the latter island retain the dextral mode of coil, while the Moorea colonies, sinistral in nature, diminished in numbers and became more closely restricted, to survive as the single association of *P. tohiveana* existing to-day. The alternative view is not tenable, namely, that *P. tohiveana* has arisen from actual migrants from Raiatea to Moorea, or that the dextral Raiatea species have come directly from sinistral *tohiveana* progenitors, transported from Moorea. The conclusion so briefly stated is supported by many bodies of evidence, which will be presented fully in the later detailed discussion of the species of Moorea.

PARTULA OLYMPIA CRAMPTON

The last species to be mentioned is another new discovery, and it is sinistral like *P. tohiveana* and *P. dendroica.* It was first found in 1919 in a high area of forest on the inward face of Mt. Mouaputa and nowhere else in Moorea (Plate 5C). Like the foregoing, it exists in unusually sparse numbers; indeed, it would seem that it is in grave danger of extinction.

Paralleling *tohiveana* in another respect, the species displays several differentiated color-types, as follows: (a) uniform fleshy-corneous (Plate 5C); (b) uniform medium brown; (c) the same with a median band of color on the apical whorls only; (d) with a vague median band over all of the whorls (Plate 5C), and (e) with two revolving bands of brown, weak and faintly-defined. Again the point of note is that the species is clearly composite as regards the factors of color, even under the identical conditions of one environment.

Some of the distinctive features recall *P. mooreana,* and it may well be that *olympia* and *mooreana* are related in ancestry. If so, the animals of this stock have completely disappeared between the small area where *olympia* is now found and the distant region of Vaianai from which *mooreana* so recently began its extension and differentiation in the ways that have been described.

CONCLUSION

The present investigation of the species of Partula living in Moorea comprises a comparison of the conditions which were found in 1907 and 1909 with what Garrett described for earlier decades, and also a second comparison of the later situations in 1919 and 1923 with my own first observations. In general terms, the changes which have occurred during the second time period are the same in nature as those which had taken place during the longer first interval.

The changes in question are of two kinds, geographical and constitutional, of which the latter are by far the most significant in connection with the problems of specific differentiation. It is by no means unimportant that exactly the same kinds of change have been demonstrated in some of the species of Tahiti.

When the several species of Moorea are brought together, they form a strikingly consistent series whose terms are mutually explanatory in various respects. *Partula taeniata* stands at the one extreme, with the widest spread and the highest degree of colonial diversification, both of which mark it as a species of long standing. This is followed by *Partula suturalis,* which has extended its range and gained greater complexity only in recent decades. *Partula mooreana* has accomplished results that are the same in principle, in even less remote years and to lesser degrees. *Partula mirabilis* is particularly significant as the next term, because its small numbers and local restriction prevented Garrett from finding it, yet to-day it rivals *mooreana* in its range and internal diversity. Near the other extreme is *Partula dendroica* whose extension has only just begun; while finally *Partula olympia* and *Partula tohiveana* exist in small single colonies and in sparse numbers, thus presenting a condition like that of other species before they had started to multiply, to diversify and to disperse more widely.

That mutation is a real and a contemporaneous process is proved in the first place by the existence of divergent individuals of adult growth, such as the two sinistral examples of *taeniata,* the dextral specimens of *mooreana* and the sinistral representatives of *mirabilis.* If clearer proof were possible, it is provided by the mutant young within the brood-pouch of a standard type of adult, as in the case of the dextral *mooreana* borne by a reversed parent.

Further summary does not seem expedient in advance of a full presentation of the facts. There are many problems that come directly into question, the one of paramount importance being that which is concerned with the possible participation of the "environment" in the stimulation of the various species to produce their novel forms; as the facts stand at present, the "environment" appears to have been negligible as regards a causal relation to the episodes of organic differentiation actually demonstrated.

THE ISOLATION OF A PATTERN VARIETY IN PIEBALD HOUSE MICE

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IN an earlier paper (Dunn, 1920) the occurrence of what appeared to be distinct pattern types of white-spotted (piebald) house mice was noted; and a preliminary description of the variations and of their behavior under inbreeding was given. The attempt to isolate these varieties has been continued, and it is the purpose of this note to report the results obtained by inbreeding two of these pattern varieties for twelve and seven generations, respectively; together with data resulting from crosses between them and from crosses of each type with the wild form.

The variations to be described arose in a random bred stock of piebald mice which were obtained from a fancier in 1919.[2]

Of the 65 piebald mice purchased a few were noted as having dorsal white spotting on the face or head only (Fig. 1) and two were noted as being spotted only in a white band around the body between the fore and hind limbs (Fig. 2). The first type was designated as *white face spotting;* the second as *belt.* These were used in other experiments involving crosses with self-colored mice. From such crosses there appeared in later generations well-defined white-faced and belted types, which were saved for further study.

The first experiment consisted of inbreeding, by brother-sister matings, the progeny of several pairs of white-faced and of belted mice. Matings between litter

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[1] Contributions in Genetics No. 26.

[2] The first two generations of the experiments to be reported were carried on at the Bussey Institution, Harvard University, and the stock was obtained through the kindness of Professor W. E. Castle.

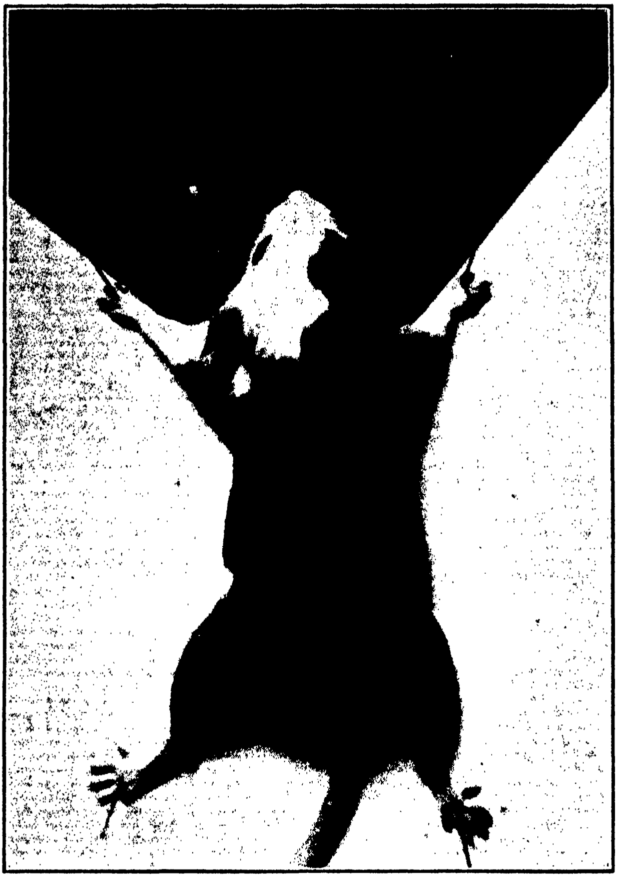

FIG. 1. The white-faced variety of piebald; a parent of one of the inbred lines.

mates were made in several lines for three generations, after which the lines which conformed most closely to the type desired were continued and the others were abandoned. In the white-faced lines, only those mice were selected for breeding which had dorsal white spotting on the face only, either as a blaze, a white nose or a wholly white face. The last type was preferred. In the belted lines selection was toward a well-defined continuous belt of white, although so few of this type were obtained that the actual criterion became "spotting in the position of

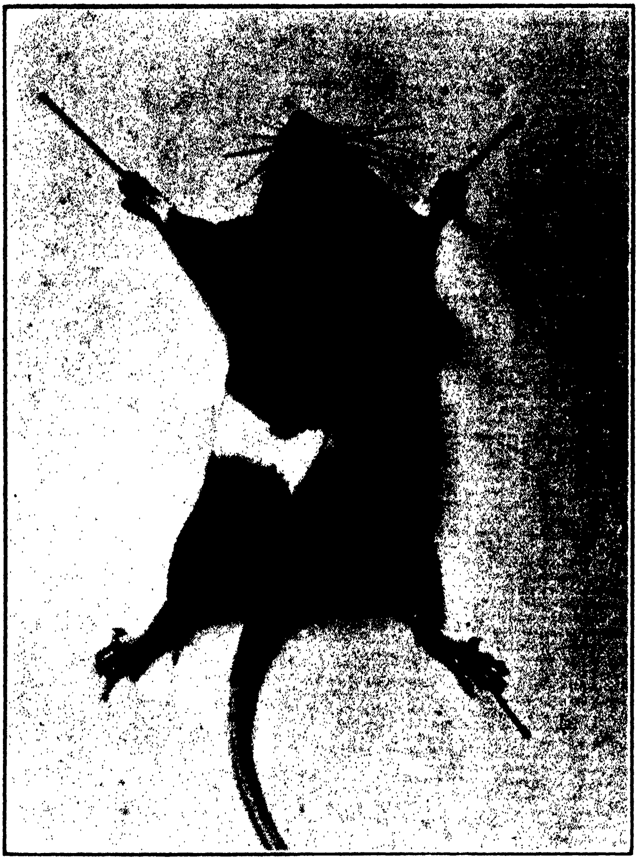

FIG. 2. A belted piebald; ancestor of one of the inbred lines.

the belt only." Shorthand descriptions of the spotting pattern and a sketch on a standard diagram were made of each mouse at about one month of age.

THE WHITE-FACED TYPE

The results of inbreeding the white-faced types are given in Table I. The P_1 generation consists of offspring of pairs selected as good white-face types. The parents were in general not closely related. These progeny were bred brother by sister to produce the F_1 inbred generation. The chief line, No. 46 of our records, is separated in the table from lines originating from other pairs. In line 46 several collateral lines were carried to the sixth generation. Thereafter all mice in this line are descended by straight brother-sister matings from one pair in the sixth generation.

TABLE I

RESULTS OF INBREEDING (BROTHER × SISTER) THE OFFSPRING OF SINGLE PAIRS OF PIEBALD MICE OF THE WHITE-FACE TYPE

Generation	Number of offspring		Offspring with white face only		Offspring with white face and other spotting	
	Line 46	All Lines	Line 46	All Lines	Line 46	All Lines
P_1	4	108	4	99	0	9
F_1	6	68	6	66	0	2
F_2	20	35	19	34	1	1
F_3	88	96	84	92	4	4
F_4	84		78		6	
F_5	32		32		0	
F_6	48		46		2	
F_7	56		56		0	
F_8	43		42		1	
F_9	33		30		3	
F_{10}	14		9		5	
F_{11}	13		12		1	
F_{12}	14		14		0	
F_{13}	17		17		0	
F_{14}	3		3		0	
	475	664	452	630	23[3]	34

It is apparent from this table that the white-faced variation bred practically true from the beginning. All the progeny of all lines were spotted on the face. In addition 34 mice out of the 664 reared were spotted elsewhere on the dorsal surface. Of these latter, 21 showed only a few white hairs or a small spot on the neck behind the ears. This appeared to be merely a fluctuation or extension of the white-face type. This leaves but 13 exceptions, which were spotted on parts other than the head or neck, and in these white hairs or small spots appeared only on the back and sides. These spots could often be interpreted as upward extensions of the ventral spotted area, which is very variable. These variations appeared to be non-heritable. Two mice with spotting on the back in addition to the face spotting produced when bred with white-faced sibs only white-faced descendants in subsequent inbred generations. Even in the tenth generation of inbreeding, five out of 14 mice showed these slight departures from the white-face type, which is added evidence that they probably occur at random in homozygous white-faced piebald mice and are non-genetic in origin.

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[3] The mice showing white spotting on other parts of the upper surface in addition to the face consisted in Line 46 of 16 with some white on the neck (partial collar), four with a few white hairs in the center of the back, one with a small white spot on the shoulder, one with a small spot on neck and one side, and one with a partial belt. In the other lines there were five with neck spotting in addition to the white face, four with small spots in the position of the belt and two with both neck and belt spotting.

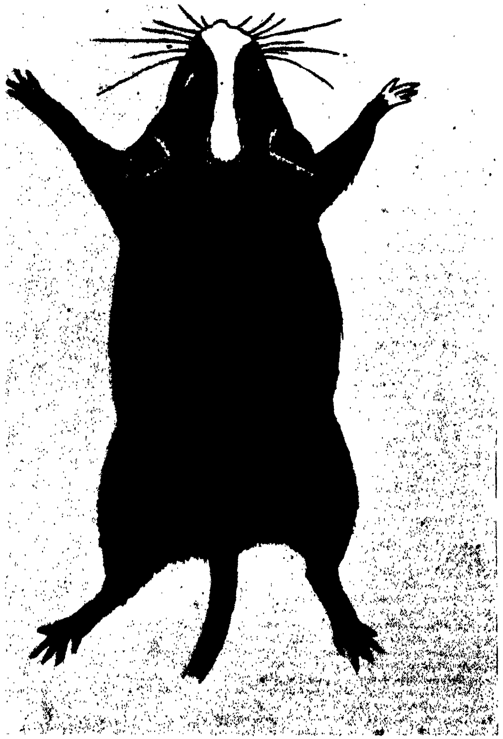

Fig. 3. A typical white-faced piebald from the 14th generation.

On the whole the white-faced type appears to be a distinct true breeding variety of piebald subject to minor non-genetic fluctuations (*cf.* Fig. 3 for present appearance of the white-faced variety).

THE BELTED TYPE

The mice selected from the random bred piebald stock as originators of belt lines all showed well-defined white belts extending across the dorsal surface between the neck and the hips and joining the ventral spotting on both sides (Fig. 2). Several pairs of these belted mice were mated regardless of relationship and their progeny used as parents of several inbred lines. Only those progeny were selected for breeding which showed some white spotting on the back between the shoulders and hips, those approaching most nearly to the true belt type being preferred. Very few true belts were obtained in any generation, and our object became to eliminate spotting elsewhere than in the mid-dorsal region.

The results of the inbreeding and selection on this criterion are given in Table II. Under "belt spotting only" are listed those mice which had dorsal white spotting in the position of the belt only. These were of various types (Fig. 4). A few had good belts; more had partial or broken or double belts and many had a small white spot in the center of the back. Under Line 55 are listed all the offspring of a single pair of P_1 mice descended by single brother-sister matings. Under "all lines" are listed several different "belt" lines through the third generation, and the collaterals of Line 55 thereafter.

The proportion of mice with spotting in the position of the belt only was very low in the early generations. In Line 55 only eight of the 29 offspring of the original belted pair showed any approach to the belt type. The others were variably spotted on back, face, head and neck regions. The only generalization we can make concerning these other types is that none showed the white-face condition alone; and that all showed spotting in the central dorsal region, accompanied by some spotting else-

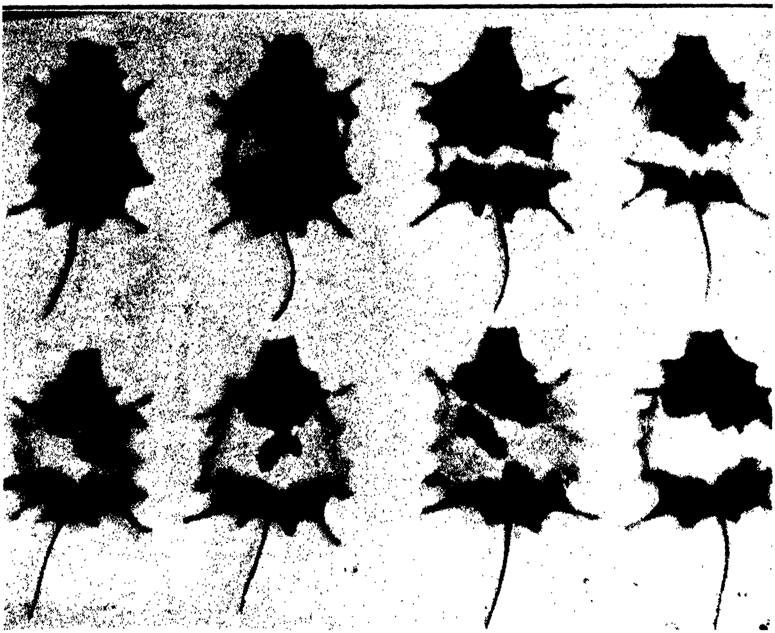

FIG. 4. Skins showing some of the variations encountered in the "belted" type of piebald. The skin of a self colored (unspotted) mouse is shown for comparison in the upper left hand corner.

TABLE II

RESULTS OF INBREEDING THE OFFSPRING OF SINGLE PAIRS OF PIEBALD MICE OF THE BELTED TYPE

Generation	Number of offspring		Offspring with belt spotting only		Offspring with belt and other spotting	
	Line 55	All Lines	Line 55	All Lines	Line 55	All Lines
P_1	29	90	8	35	21	56
F_1	15	122	8	62	7	60
F_2	19	88	15	49	4	39
F_3	13	36	11	31	2	5
F_4	47	77[4]	39	65	8	12
F_5	27	48[4]	25	40	2	8
F_6	28	58[4]	27	51	1	7
F_7	19	48[4]	19	47	0	1
F_8	26	26	25	25	1	1
	221	593	177	404	44	189

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[4] Collaterals of Line 55.

where. The proportions of belt types increased and the proportions of other types decreased under inbreeding, until in the seventh generation only one out of the 48 mice showed spotting elsewhere than in the belt position. It can not be said, however, that we have produced a true breeding belted type of piebald mice, for the proportion of typically belted mice is still low after seven generations of inbreeding and there is great variability in the amount and position of the dorsal spots (Fig. 4). The most that can be claimed is that inbreeding accompanied by selection has produced a type which is not spotted on the face or elsewhere than in the central dorsal region. These results are, therefore, somewhat at variance with those obtained in the white-face inbreeding, since the white-face type was easily isolated and fixed and appeared therefore to rest on a relatively simple genetic basis, while the true belt pattern appears to be either a random expression of diffuse mid-dorsal spotting or to depend on a more complex genetic basis.

From the results of both inbreeding experiments it may be concluded that some of the variations in the spotting patterns of piebald mice are genetic, and that two of them—the white-face, and the mid-dorsal non-white-face type—have been isolated by inbreeding.

AMOUNT OF SPOTTING IN WHITE-FACE AND BELTED TYPES

All mice recorded in the above experiments were described also with respect to the amount of dorsal white spotting. The same percentage scale described in a previous paper (Dunn, 1920) was used. The results for the two chief inbred lines are given in Table III.

The average percentage of the dorsal surface which was white was much less in the white-faced than in the belted parents. The averages for the white-face P_1 and F_1 generations are based on too small numbers to be reliable. After the second generation the amount of white spotting in this line declined quite steadily.

This apparently represents a real diminution in the amount of white spotting and not a change in the meth-

TABLE III

AVERAGE AMOUNT AND VARIABILITY OF DORSAL WHITE SPOTTING OF TWO INBRED LINES OF PIEBALD MICE

	Line 46 (White Face)				Line 55 (Belted)			
Generation	N.	Mean per cent.	S. D.	C. V.	N.	Mean per cent.	S. D.	C.V.
P_1	4	9.5	4.9	51.6	29	17.3	7.7	44.5
F_1	6	14.3	1.9	13.3	15	18.0	9.3	51.7
F_2	19	13.4	6.6	49.3	19	22.0	12.5	56.8
F_3	88	12.0	3.7	30.8	13	13.9	12.8	92.1
F_4	84	10.0	3.9	39.0	47	15.8	11.4	72.1
F_5	32	10.3	3.4	33.0	27	10.1	7.1	70.3
F_6	48	9.8	4.2[5]	42.9	28	13.6	8.1	59.5
F_7	56	7.5	2.6	34.7	19	15.9	7.3	45.9
F_8	43	7.0	2.6	37.1				
F_9	33	6.7	2.5	37.3				

ods of estimating the size of the white areas. This is supported by comparison of the white-faced mice of the present (fourteenth) generation with photographic records of those in the parental and early generations (*cf.* Figs. 1 and 3) and by checking the numerical estimates against the standard sketch record which we made for each mouse at weaning time. The selection practiced was intended to fix a pronounced and extensive white face in this type, but we were also influenced by a desire to avoid extension of the spotting to the neck or other adjacent regions. The latter circumstance and the chance selection of a line with an inherent tendency to show but little spotting on the face probably accounts for much of the decline noted. Changes in the amount of white spotting were most marked in the white-faced line, the belted line varying irregularly from generation to generation.

Variation in the amount of spotting in these inbred mice is considerably less than in the random stock of piebalds from which they are descended. The coefficient of variation of 473 stock piebalds was about 88 per cent.

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[5] Variability increased by a single aberrant individual with 26 per cent. of white spotting due possibly to mutation.

(Dunn, 1920, p. 483), while for the white-faced line it is less than half as great and for the belted line about two thirds as great. These lines are, therefore, genetically different from random bred piebalds not only in localization, but in amount of spotting as well. Further inbreeding appears not to have reduced further the variability of spotting in the white-faced line. The standard deviation (Table II) appears to have decreased under inbreeding, but that this is due entirely to decrease in the mean amount of spotting is shown by the fact that the coefficient of variation has shown no significant change under inbreeding. Variations in the small amount of spotting occurring within the inbred white-faced line appear, therefore, to be non-genetic. In the belted line, although the numbers are small, variability appears to have declined after the third generation, indicating that some of the variations in the larger amounts of spotting characteristic of this line are produced by heritable factors. Consideration of these data and observation of the animals indicate that the white-face line has now (fourteenth inbred generation) reached the limit of reduction in variability; and that the variability remaining (between 30 and 40 per cent.) measures the amount of non-genetic variability occurring in the expression of the piebald gene.

CROSSES INVOLVING WHITE-FACED AND BELTED MICE

The cross of belted and white-faced types has been made reciprocally at three different stages of inbreeding. The reciprocal crosses have given substantially the same results. A cross when the belt and white-face lines were in the second-third generations produced an F_1 generation consisting of twelve white-faced piebalds. These inbred produced a second generation of 52 white-faced, 16 belted, two with both white face and belt spotting and three with no dorsal spotting whatever. A backcross of F_1 mice to the belted parent produced eight white-faced, four belted, one white-faced and belted and two with no dorsal spotting. A cross in the fifth-seventh inbred generation produced 24 white-faced, two with white face and belt and three with no dorsal spotting. These when inbred produced in F_2 25 white-faced, 20 with white-face and belt spotting, six belted and two with no dorsal white. A cross in the twelfth generation has produced two white-faced, seven with no dorsal spotting, three with white face and belt and two with belt only. No second generation has been reared from the last cross, since the variation in evidence in the first generation indicates that one or both of the parents were not homozygous or that other factors have entered to complicate the results. Adding together the results of the first two crosses gives the following totals:

CROSS OF WHITE-FACE AND BELT PIEBALDS

	White Face	Belt	White Face and Belt	No Dorsal Spotting
F_1	36	—	2	3
F_2	77	22	22	5

It is apparent that in these crosses the "white-faced" condition behaved as a dominant in inheritance and that in the second generation it segregated from the alternative condition "no white spotting on the face" in a majority of the cases. The ratios obtained are discussed in a later section.

CROSSES WITH THE WILD TYPE

As additional tests of the inheritance of the white-faced and belted conditions we have crossed both kinds of mice with the same strain of wild house mice. The wild mice were trapped in a barn at a considerable distance from any domesticated mice, and have never shown any evidence of intermixture with domesticated varieties. The mice of this strain have been invariably self-colored with the black agouti coat which is typical of the wild form. The cross of white-faced piebald mice from the fifth-seventh generations of Line 46 with the wild stock produced in F_1 only black agouti self-colored mice of the wild type. In F_2 of the 45 piebald mice which appeared as recessive segregates all except three were spotted on the face only in the white-faced pattern. In the three exceptions (which occurred in the offspring of one F_1 mother) the white spotting of the face extended backward between the ears and over one or both shoulders in the form of a partial collar. One of these when bred back to his F_1 self mother produced six self and seven piebald young. Of the piebalds three showed the ordinary white-faced spotting, while four reproduced the extended spotting of the father, indicating that the genotype of this female included factors which tended to increase the amount of white in her piebald descendants. The white-faced pattern has, therefore, been passed through a wild self-colored race and extracted in its original condition with the exception noted.

Individuals of the "belted" line (No. 55) crossed with the wild race produced all wild type self mice in F_1 and in F_2 of the 37 piebalds born, 23 were of the belt type, nine had both belt and white-face spotting, three had white-face spotting only and two had no dorsal white spotting. The belted parents used were true belts from the early (F_1 and F_2) generations of inbreeding and the proportion of offspring from the wild cross showing white-face in addition to belt spotting is about the same as occurred in pure belt matings. We do not believe the tendency for white spotting to be exhibited principally in the belt region has been altered by the cross. One of the F_2 white-faced mice out of this cross was bred to a sister with belt spotting and a small white blaze between the eyes. Of their 26 offspring, nine were of the belt type, 16 had both blaze and belt spotting, and one had no dorsal spotting. No mice with only white-face spotting appeared, so that the small amount of white-face spotting in this mouse appeared to be distinct from the usual type of white face and had probably not been introduced by crossing with the wild type.

DISCUSSION

The evidence from the inbreeding of white-faced piebald mice and from crossing them with non-white-faced piebalds and with wild mice leaves little doubt that "white-face" is a distinct true-breeding variety of piebald. It appears to contain a gene (or genes) for the restriction of white spotting to the head and face, and is therefore expressed only in the presence of the gene for piebald or recessive white spotting. Aside from the probability that this modifying gene acts as a dominant little is known of its inheritance. It has been passed through self-colored mice and recovered without any significant alteration. It has shown evidence of segregating from crosses of white-faced by "belt" mice, although the F_2 ratios obtained from this cross are inconclusive because of the appearance in the second generation of a number of mice with *both* white-face and "belt" spotting. Such mice have frequently appeared in the progeny of "belt" parents and may contain recessive modifying genes distinct from those found in the true breeding white-face lines. When bred, such "white-face and belt" mice have not behaved as ordinary hybrids between white-face and "belt" types, since they have never produced mice with white spotting on the face only. With only the present evidence at hand they can not be satisfactorily explained. It is otherwise with the other type encountered, *viz.*, mice with no white spotting on the dorsal surface. These have frequently occurred in many forms of piebald and apparently contain modifying genes which reduce the general *amount* of white spotting (Dunn, 1920). They are probably very dark piebalds which are unable to express any dorsal spotting pattern and are thus similar to the dark piebald (hooded) rats obtained by Castle (1916).

We are unable to make any final statements concerning the "belt" pattern. This has not become fixed under inbreeding, except that it does not produce mice of the white-face type.

CONCLUSION

The isolation of at least one true-breeding pattern variety from a variable stock of piebald mice demonstrates that a portion of the great variability in piebald spotting is due to genes affecting the *distribution* or localization of spotting in the coat. Experiments reported previously (1920) indicated that other genes are responsible for a part of the variability in the general *amount* of spotting. It is probable, then, that the various types of piebald mice owe their origin in part to heritable variations in other genes modifying the expression of the principal gene concerned with recessive white spotting.

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THE RELATION BETWEEN PREVIOUS SEXUAL REPRODUCTION AND THE PRODUCTION OF MALE OFFSPRING IN MOINA MACROCOPA[1]

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WEISMANN ('79) set forth the hypothesis that in Cladocera there is an innate sexual cycle quite independent of environmental influences. Later workers have taken various attitudes toward this theory ranging from (a) complete acceptance, (Keilhack, '06; Kuttner, '09; van Herwerden, '18); through (b) a modified acceptance—belief in an internal sexual cycle which, however, is more or less subject to environmental modification or control (Issakowitsch, '08; Woltereck, '09; McClendon, '10; Papanicolau, '10; Grosvenor and Smith, '13; Scharfenberg, '14); to (c) a belief in the direct opposite of Weismann's theory—that sexual manifestations in Cladocera are not influenced by internal factors but are called forth solely by environmental influences.

Weldon ('09, p. 50) by inference suggests and possibly he meant to subscribe to this last view. Later the writer ('13, '14) and Agar ('14) came out squarely against the Weismannian hypothesis. The writer in his first report had reared thirteen lines of Daphnia for from 25 to 33 parthenogenetic generations. In the second paper ('14) the writer reported 100 parthenogenetic generations of *Daphnia pulex* and 76 generations of *Simocephalus.* Agar based his conclusions upon the rearing of *Simocephalus vetulus* for 46 generations by parthenogenesis and without the occurrence of degenerate forms or of more than the sporadic occurrence of males. Later ('15) the writer published a brief note stating the results of an experiment which showed that the reproductive vigor of old laboratory lines, parthenogenetic for 127 generations, was substantially the same as that of newer lines just brought into the laboratory.

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[1] Assisted by Kathleen Gavin.

At the present time stocks of several species of Cladocera, long in the laboratory and exclusively parthenogenetic for long periods of time (some more than 500 generations), show no reduction in vigor or proneness to produce either sexual forms or abnormalities. Such histories seem inconsistent with an innate sexual cycle.

We have recently hatched young (females) from fertilized (sexual) eggs of *Moina macrocopa.*[2] If there were really an internal sexual cycle in any sense of the term, it would seem that as parthenogenesis continued the stock would become more and more prone to produce males and ephippial (sexual) eggs; or at least that strains just derived from sexual eggs should be less readily caused to go into sexual reproduction than strains parthenogenetic for many generations.

In order to get evidence on this point two stocks were utilized. The one was from the parent line of *Moina macrocopa* (Line 1012) in its 294th to 301st generation of laboratory descent solely by parthenogenesis. The other was stock in its 1st to 7th generation of descent by parthenogenesis from lines derived by sexual reproduction from Line 1012. These two stocks were simultaneously subjected to environmental conditions known to call forth the production of males. The means employed to cause the production of males is the simple expedient of crowding the mothers in the culture bottles (cf. Grosvenor and Smith, 1913; Banta and Brown, 1923). Since environmental influences are utilized, it is desirable to set forth some of the details of the conditions of the experiments.

In preparation for the first of these experiments (Experiment 111, started January 14, 1922) newly released parthenogenetic young of stock in its second parthenogenetic generation and newly released young stock in its 293rd[8] consecutive parthenogenetic generation were isolated in similar culture bottles on the same day, given the same food and otherwise treated as nearly alike as possible. When these produced young, large broods which had been released from the mothers' brood-chamber near the same time were taken for use in starting the experiment. Hence, all the broods of young employed as mothers in an experiment were within an hour or two of the same age and were from mothers which had been subjected to identical treatment from birth. In addition to the care given the grandmothers and the pains taken in the selection of the mothers, every effort was made to accord identical treatment to all the material concerned in an experiment. All the future mothers were placed in the culture bottles at the same time; an equal amount (75 cc) of culture water, from the same culture jar, was placed in each bottle; and the individuals were in every way treated alike, except that some from each brood of both new and old parthenogenetic stocks were crowded —10 in a bottle—while the others were in individual bottles.

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[2] These ex-ephippial females themselves produced males among their offspring.

In Experiment 111 three broods from the third generation of three new strains of *Moina macrocopa* were used in making up the 8 crowded and the 10 uncrowded bottles of the new stock; while two broods of the 294th generation of Line 1012 provided the material for the 4 crowded and the 6 uncrowded bottles of the old stock. The data of this experiment are shown in Table 1, in which the numbers of female and male young produced in each bottle are indicated by broods.

From inspection of this table it may be seen that the uncrowded mothers of the new stock produced 0, 7.7, 37.1, and 0 per cent. males in their first to fourth broods, respectively, or 12.4 per cent. males for the total for the four broods (1,113 young); while the old stock produced 0, 30.9, 0 and 28.7 per cent. males for the first four broods, respectively, or 13.6 per cent. males for all (580 individuals). While there is considerable variation by individual broods, the percentage for the entire four broods for the new and old stocks are nearly the same—12.4 per cent. and 13.6 per cent. The percentages of males in the first, second and third broods in crowded bottles (81 mothers in 8 bottles) of the new stock are 24.0, 64.6 and 40.4 or for the lot (2,205 ♀s and 1,486 ♂s) 40.3 per cent. These compare with corresponding percentages of 19.5, 73.7 and 27.6 for the different broods and 37.1 per cent. for all the broods (1,185 ♀ and 700 ♂s) of the old stock (39 mothers in 4 bottles).

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[8] It was the 293rd *laboratory* generation. We do not know when sexual reproduction may have occurred previously to the taking of this stock into the laboratory.

In spite of variations the percentages of males produced in this experiment by the new and the old stocks (both by crowded and uncrowded mothers) are so nearly the same as to indicate no significant difference in the incidence of males between stock which had experienced sexual reproduction 3 generations previously and stock which had last passed through sexual reproduction at least 294 generations previously.

Two other similarly extensive experiments were conducted with new and old parthenogenetic stock. In one of these experiments new stock in the 7th parthenogenetic generation and in the other case stock of the first parthenogenetic generation was employed. Brief summaries of the data for these experiments, together with that for Experiment 111 (detailed in Table 1) are given in Table 2. This table consists of four portions, the first and second of which show summaries of data for new and old uncrowded bottles and the third and fourth for new and old stock in crowded bottles.

Of the 1,865 young produced by mothers of the new stock in uncrowded bottles in these three experiments 178 or 9.54 per cent. were males. Of the 1,460 young produced by similarly treated old stock 147 or 10.07 per cent.

TABLE I.—DATA OF EXPERIMENT 111

New *Moina macrocopa*. Lines 1133, 1134, and 1135. (3rd generation).

Line No.	Bottle No.	Uncrowded Mothers								Crowded Mothers						
		First Brood		Second Brood		Third Brood		Fourth Brood		First Brood		Second Brood		Third Brood		% ♂'s
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	
1133	1	17	0	32	0	0	34	24	0	157	35	23	100	94	34	38.1
	2	17	0	27	0	0	28	31	0	163	41	59	85	75	52	37.5
	3	23	0	34	0	28	0	31	0	131	45	37	95	61	55	46.0
1134	4	21	0	34	0	0	27	32	0	111	107	75	31	87	53	41.2
	5	22	0	29	0	32	0	34	0	188	49	59	63	64	53	34.7
	6	23	0	35	0	3	26	33	0							
	7	20	0	29	2	32	0	28	0							
1135	8	15	0	27	0	41	0	37	0	174	27	31	132	79	52	42.6
	9	20	0	0	21	28	0	32	0	183	13	23	114	97	25	33.4
	10	17	0	30	0	31	0	26	0	118	70	72	71	44	84	49.0
Totals		195	0	277	23	195	115	308	0	1225	387	379	691	601	408	
Average Brood		19.5		30.0		31.0		30.8		19.9		13.5		12.9		
Percent ♂'s		0.0		7.7		37.1		0.0		24.0		64.6		40.4		
Total young		1113								3691						
Average brood per mother		27.8								15.5						
Average percentage ♂'s		12.4								40.3						

TABLE I—*Continued*

Old Moina macrocopa. Line 1012. (294th generation.)

1012	1	25 0	Died			212 8	17 142	110 13	32.5
	2	20 0	35 0	32 0	33 0	132 19	35 93	88 12	32.7
	3	25 0	3 0	34 0	0 37	93 124	72 37	49 131	57.7
	4	27 0	18 11	33 0	3 0	199 3	15 118	163 0	24.3
	5	26 0	0 31	32 0	37 0				
	6	27 0	38 0	34 0	19 0				
Totals		150 0	94 42	165 0	92 37	636 154	139 390	410 156	
Average Brood		25.0	27.2	33.0	25.8	20.3	14.3	15.3	
Percent ♂'s		0.0	30.9	0.0	28.7	19.5	73.7	27.6	
Total young			580				1885		
Average Brood per mother			27.6				16.7		
Average percentage ♂'s			13.6				37.1		

were males. Similar comparisons of the total numbers of young from crowded mothers of all three experiments shows that the new stock produced 4,571 females to 2,735 males or 37.43 per cent. males, while the old stock produced 3,628 females to 2,169 males or 37.42 per cent. males. Hence, totals of substantial numbers of young (16,428 young from 323 mothers) from new and from old stock reared both in crowded and in uncrowded conditions show approximately the same percentages of males for the new and for the old stock.[4]

Further examination of these experiments serves to give additional emphasis to this similarity of male production in new and old parthenogenetic stocks. Individual experiments show fairly close agreement in male percentage between the new and the old stock, whether the comparison is made between uncrowded or crowded stock. Experiment 111, already considered, indicates this. Comparing similar figures for Experiment 112: the new stock, uncrowded, produced 7.3 per cent. males; the old, 10.4 per cent. males; and in crowded bottles the new stock produced 37.2 per cent. and the old 46.3 per cent. males. In Experiment 117 the uncrowded stock died and could not be followed beyond the first broods. The crowded mothers produced 31.9 and 27.2 per cent. males in the new and the old stocks, respectively. Hence, in these experiments all the comparisons of male production between new and old stocks, whether isolated or crowded, show substantially the same percentages of males.[5]

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[4] Undoubtedly the remarkably close agreement in these percentages, 37.43 and 37.42, is a coincidence. One would not expect a second extensive series of such experiments to give so nearly precisely the same percentages, though they would not be significantly different.

[5] Certain points of minor interest are suggested by further examination of Table 2, although their significance is not so clear.

(1) The uncrowded new stock in both Experiment 111 and Experiment 112 produced fewer males in second broods than the old stock (although the reverse was true for third broods); while in the crowded bottles (in which male production begins in first broods) the new stock produced a larger percentage of males in all three experiments in first broods than the old stock.

On two occasions we hatched a number of ex-ephippial young *Moina macrocopa* on the same day. Although we did not then have at hand newly released parthenogenetic young of the old stock to use in conducting a comparative experiment, such as those discussed above, we crowded the newly hatched (ex-ephippial) young to see if they would produce a considerable percentage of males. In Experiment 118 (March 4, 1922) ten such crowded mothers produced 50 ♀s and 53 ♂s in their first broods and 34 ♀s and 8 ♂s in second broods. This represented male percentages of 51.5 and 19 for the first and second broods, respectively, or 42.1 per cent. for the two broods considered together. In Experiment 126 (September 6, 1922) 14 ex-ephippial mothers were crowded in a single bottle and in their first broods produced 36 ♀s and 76 ♂s or 67.9 per cent. males.

While these last two experiments contain only a little data, they are of interest, inasmuch as they represent two separate lots of material and were hatched at different times. In crowded bottles they produced characteristically high percentages of males in their first broods, regardless of the fact that they had just hatched from sexual eggs.

Hence, not only new stock in the 1st, 3rd and 7th parthenogenetic generations but even those individuals, themselves, hatched from sexual eggs produced (under appropriate conditions) as large percentages of males as stock which had not had sexual reproduction for 300 or more generations.[6] It would, therefore, seem clear that there are no significant differences in male-produc-

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(2) The new stock, both in crowded and uncrowded bottles, produced smaller first broods than the old stock. The six differences (between the uncrowded new and old stocks and between crowded new and old stocks in three separate experiments) are all differences in the same direction. This can scarcely seem a mere chance occurrence.

[6] The five experiments discussed are not selected experiments. On the contrary, they represent all the experiments conducted to date in testing the male production of stock which had recently had sexual reproduction. There is every reason to believe that these results are typical and that like experiments would at any time give similar results.

TABLE II—SUMMARY OF DATA OF EXPERIMENTS 111, 112, AND 117

(Started Jan. 14, Feb. 2, and March 3, 1922, respectively.)

Comparison of Male Production in New and Old Parthenogenetic Stocks of *Moina macrocopa*.

Uncrowded New Stock

			First Brood				Second Brood				Third Brood				All Broods			
Exp. No.	Par. Gen.	No. Bot.	♀	♂	Av. Br'd.	Av % ♂s	♀	♂	Av. Br'd.	Av. % ♂s	♀	♂	Av. Br'd.	Av. % ♂s	♀s	♂s	Av. Br'd.	Av. % ♂s
111	3	10	195	0	19.5	0	277	23	30.0	7.7	195	115	31.0	37.1	975	138	27.8	12.4*
112	7	6	140	0	23.3	0	212	1	35.3	0.5	158	39	32.8	19.8	510	40	30.6	7.3
117	1	8	177	0	22.1	0	25	0	25.0	0.0	Died				202	00	22.4	0.0
Totals and Averages		24	512	0	21.3	0	514	24	31.6	4.4	353	154	31.6	30.3	1687*	178	27.8	9.54
Uncrowded Old Stock																		
111	294	6	150	0	25.0	0	94	42	27.2	30.8	165	0	33.0	0.0	501	79	27.6	13.6*
112	298	8	196	0	24.5	0	228	29	32.1	11.3	159	39	33.0	19.7	583	68	29.6	10.4
117	306	8	204	0	25.5	0	25	0	25.0	0.0	Died				229	0	25.4	0.0
Totals and Averages		22	550	0	25.0	0	347	71	29.8	16.9	324	39	33.0	10.7	1313*	147*	28.0	10.07

TABLE 2—*Continued*

Crowded New Stock

			First Brood				Second Brood				Third Brood				All Broods			
Exp. No.	Par. Gen.	No. Bot.	♀	♂	Av. Brd.	Av. % ♂s	♀	♂	Av. Brd.	Av. % ♂s	♀	♂	Av. Brd.	Av. % ♂s	♀	♂	Av. Brd.	Av. % ♂s
111	3	8	1225	387	19.9	24.0	379	691	13.5	64.6	601	408	12.9	40.4	2205	1486	15.5	40.3
112	7	4	386	426	21.4	52.5	459	85	14.7	15.6	300	167	15.1	35.8	1145	678	17.2	37.2
117	1	4	751	122	21.8	14.0	224	114	9.3	33.7	246	335	16.1	57.7	1221	571	16.0	31.9
Totals & Averages		16	2362	935	20.74	28.4	1062	890	12.84	45.6	1147	910	14.18	44.2	4571	2735	16.02	37.43

Crowded Old Stock

			First Brood				Second Brood				Third Brood				All Broods			
Exp. No.	Par. Gen.	No. Bot.	♀	♂	Av. Brd.	Av. % ♂s	♀	♂	Av. Brd.	Av. % ♂s	♀	♂	Av. Brd.	Av. % ♂s	♀	♂	Av. Brd.	Av. % ♂s
111	294	4	636	154	20.3	19.5	139	390	14.3	71.0	410	156	15.3	27.6	1185	700	16.7	37.1
112	298	4	582	321	23.2	35.5	195	326	13.7	62.6	360	335	18.8	48.2	1137	982	18.6	46.3
117	306	4	953	52	25.1	5.2	53	370	10.6	87.5	300	65	9.1	17.8	1306	487	14.9	27.2
Totals & Averages		12	2171	527	22.86	19.5	387	1086	12.8	73.7	1070	556	14.26	34.1	3628	2169	16.7	37.42

*The data for "all broods" of the uncrowded stock of Experiment 111 include data for fourth broods which are given in Table 1, but not in this Table.

ing propensity between stocks which have recently had sexual reproduction and stocks which have undergone parthenogenetic reproduction exclusively for a long series of generations.

The facts would seem to warrant the conclusion that exclusively parthenogenetic reproduction for an indefinite number of generations does not modify the tendency to produce males in *Moina macrocopa.*

The former evidence against an innate sexual cycle in Cladocera, *i.e.* (1) that parthenogenetic reproduction may be continued for hundreds of generations, and presumably indefinitely, without the intervention of sexual reproduction and (2) that sexual forms may be called forth at will in many (at least) forms of Cladocera, is now supplemented by the results of the experiments here recorded which indicate that stock recently derived from sexual eggs is no less (or more) prone to produce males under appropriate environmental conditions than stock which has descended 300 generations since last undergoing sexual reproduction.

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STATION FOR EXPERIMENTAL EVOLUTION

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DO THE CHARACTERISTICS OF PREHISTORIC HUMAN REMAINS IMPLY A COMMON ANCESTRY FOR MAN AND APES?

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SINCE resemblance to the apes increases as we trace back man's ancestry into neolithic times, the later paleolithic, the earlier paleolithic and those still earlier stages represented by the remains from Heidelberg, Piltdown, Java, this resemblance which intensifies with age has been accepted as pointing to a common ancestry for man and apes. There may be other abundant evidence for supposing such a common ancestry, but we believe the evidence of prehistoric human remains does not justify that inference, though of course it does not discountenance it. We base this conclusion on the fact, if fact it be, that practically all the changes in man's structure traceable through prehistoric remains are the result of changes in food and habits. Let us see what these changes are and what shifts in man's diet or habits might account for them.

The most notable changes are those in the skull. Briefly, the story of change from prehistoric to modern man is: A higher frontal region; increased bregmatic height; smaller superciliary ridges; increased head width; less facial projection; lessened height of orbits and a shifting of the transverse diameter downward laterally; a more oval palate; smaller teeth; diminished size of third molar; less specialization in type of teeth; shorter, wider, and more ovoid mandible; increased chin prominence; smaller ramus; decrease in size of condyles; decrease in distance between condyle and coronoid process; in general a greater smoothness, less prominent bony protuberances, less of the "savageness" of appearance which is to be noted in apes.[1]

Though there is evolution in type this evolution is result rather than cause. The change in type is notable, but we believe there is reason to assign it to change in function, to use and disuse.

Practically all the above-mentioned features of the skull are intimately linked together, so that scarcely can one change without change being reflected in the others, some, of course, reflecting the change more immediately and markedly than do others. If we suppose that man's diet and his manner of preparing food have changed, we have an index to most of these skull changes, provided the change has been from uncooked or poorly cooked food to better cooked, from more stringent diet to less stringent. A considerable development of the muscles concerned with chewing will bring about the type of changes which we find as we push human history further and further into the past.

It will affect most notably the region in which these muscles immediately function. With tough food and large chewing muscles is associated a large mandible with broad ramus, large condyles, heavy bony tissue. The larger teeth are accommodated to the tougher food and their greater specialization is a form of adaptation to the needs of the masticator. As larger teeth demand more alveolar space, we find elongated alveolar regions with greater sagittal diameter, giving the more prognathous and more angular mandible. The increased width of the ramus is a mechanical advantage in the leverage which the mandible must perform in chewing food, while the larger condyle affords a better resisting fulcrum and is associated with the greater side-to-side play which goes with longer mandible and the chewing of tougher food.[2]

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[1] Regarding changes in chin region see Wallis, Wilson D., "The development of the human chin." *The Anatomical Record,* Vol. 12, No. 2, March, 1917, 315–328.

[2] Hawkes, E. W., and Wallis, W. D., "Notes on the Glenoid Fossa." *American Anthropologist,* Vol. XVIII (1916), 440–446.

The more forward projection of teeth in both upper and lower alveolar region is in accordance with what we find in animals which use the teeth for the mastication of tough food and no doubt is a function of mastication. The palate conforms to the mandible, with which it forms a physiological unit, however separate morphologically the two may be, hence is long and less arched. Zygomatic arches stand out for the accommodation of the large chewing muscles which pass beneath them. The adjacent walls of the skull are flattened and forced inward by the pull of muscles which of necessity is inward as well as downward, giving us an elongated skull. The temporal muscles reach far up on the skull, giving rise to a high temporal ridge; they extend forward as well as backward, giving a more prominent occipital region and a more constricted forward region, resulting on the forehead region of the skull in the elevation of the superciliary ridges and intervening glabellar region.

The facial region is constricted laterally and responds in a greater forward projection, one result being that the transverse diameter of the orbits is thrust upward outwardly, giving the horizontal transverse diameter which characterizes the apes and which is approximated in prehistoric man and some contemporary dolichocephalic peoples. In young anthropoid apes, when the chewing muscles are little developed and there is little constriction in the region posterior and inferior to the orbits, the transverse diameter of orbits is oblique, as in man, being elevated to the horizontal when temporal muscles develop and function more vigorously, thrusting in and upward the outer margins of the orbits. Constriction of outer margins of orbits gives the high orbits which we find in apes and in prehistoric human types.

Elongation of the skull increases the distance between bregma and nasion and gives the low retreating forehead and low head-breadth-height index.

That muscular pull has this result is indicated by the laboratory experiments of Arthur Thomson, conducted on inflated canvas bags of the shape of a skull, with attachments corresponding to the chewing muscles and with variations in the pressures and pulls applied. It is further indicated by the fact that the Eskimo, a people living on raw food, have almost all the "primitive" characteristics in a more pronounced degree than do other contemporaneous peoples. Again, in the Australians, a people whose cooking of animals has attained little development—they cook the animals whole over an open fire—we find these "primitive" features. On the other hand, I am not aware that these conditions prevail among the negroes, who constitute a third group exemplifying these "primitive" traits.

As to other skeletal characters we have no evidence for the earliest remains, excepting only the femur of Java man, though there is abundant material from the much later, though still early, Neanderthal and Cro-Magnon types. Here the most notable differences have to do with the flexure of the knees and the larger posterior diameters of the lumbar vertebrae, both ape-like characteristics. A stooping posture can be inferred from the shift in plane of articular surfaces at the head of the femur and on the lower end of the femur, the upper articular surface of the tibia and the articulation of the tibia with the subjacent malleolus. That these differences exist is clear, but that they have evolutionary significance beyond reflecting change in form associated with change in function is not clear. We find them commonly in contemporary peoples of the lower cultures, such as Africans, Australians and others. The explanation of them is to be found in the absence of chairs in these cultures. The position of rest is that of squatting on the heels, or of sitting on the haunches with knees flexed, or other pose than that which Europeans assume when they sit. This throws the articular surface of the head of the femur further forward, throws back the articular surfaces of the lower end of the femur and the upper end of the tibia, and throws forward the articular surface at the inferior end of the tibia and on the subjacent malleolus. The greater posterior diameter and lessened anterior diameter of the vertebrae of the lumbar region are a function of this more frequent and forcible bend forward of the vertebral column. Similar differences are found in savage tribes whose culture lacks chairs.

If the above interpretations are correct, it follows that a return to the conditions of diet and of life which characterized prehistoric man would be followed by a return to the type of prehistoric man. Yet from this transition to type more simian one could not say we were approaching a common ancestor, for, if we have one, we would be getting further away, no matter how similar the type might become.

It would be a case of similarity due to similar conditions, a convergent evolution, not a similarity due to the transmission of qualities from some common ancestor of a remote past. If this be true, it is equally true that an increase in similarities as we push back the time period does not imply common ancestry, supposing those changes are due to changes in function, to function related to diet and posture of body. Since the male of the human species resembles the anthropoid ape in nearly all these characteristics more than does the female, when we take male and female of a given group—though of necessity both sexes must be equally remote from simian-like ancestry—it seems clear that mere resemblance can not constitute an argument for phylogenetic descent. These sex differences, moreover, are in support of what we have said above, seeing that the more muscular male takes on those same simian attributes, though to a lessened degree, which are characteristic of early man. If he is more conservative of the type—though this attribute usually is assigned to the female—this is because his bodily activity is more nearly that of prehistoric man and that of his supposed relatives, the anthropoid apes.

Though this is not a critique of the theory of evolution but merely of one line of argument wherein change of type is assumed to show common ancestry with a zoologically similar genus, we would like to point out that man, if descended from an ancestor common to apes, should in type more nearly approach that remote ancestor as we go back to earlier simian types, whereas we commonly insist that the earlier human types approximate contemporary simiae. It would seem to be of as much importance to the theory of common ancestry to show that earlier simian forms approach the type of earlier human forms. Yet it seems that they do not so approach them. The resemblances of prehistoric man hark forward to modern apes rather than back to prehistoric anthropoid ancestry. Prehistoric anthropoid forms seem to help us as little in supplying the missing link as do those prehistoric forms on which we have placed too much reliance, because an age with its mind made up to evolution of a unilinear type has seen what it has looked for. In unravelling the past can we do other than follow the methods of the geologist, who infers past changes by a study of existing forces and infers the existence of no force with which he is unacquainted? If we are correct in our interpretation, in so far as prehistoric human remains are concerned, it is not so much evolution which has given us modern man, as man who has given us his type by evolving it through physiological or functional changes growing out of changes in his culture, an evolution which he is still continuing. If the cause lies within the species it does not imply common ancestry with a morphologically and anatomically similar species, even as it is not an argument against such common ancestry.

What, then, is our conclusion with regard to the relationship between man and apes? Briefly this: A review of the similarities in structure, in blood and in use of the limbs, points to the apes as man's nearest relatives in the animal kingdom, his first cousins, if he has any. That some creature is his nearest relative is a conclusion to which we are driven by a consideration of the facts of zoological life. As regards prehistoric human remains we do not find that the increasing resemblance to apes as we go back in time implies simian ancestry, seeing that these changes may be due to changes in food and posture, representing the acquisition of form growing out of function or closely correlated with function. In that case, man's increasing resemblance to apes has other explanation than descent from a common ancestor, being, if our interpretation is correct, a case of convergence, the response of similar form to similar function. As a matter of fact, the increase from long-headedness to short-headedness, which we find as we go from earliest man to more recent man of the prehistoric past, is a proceeding to greater resemblances with the apes. Round-headedness is a characteristic of apes no less than, in fact much more than, it is a characteristic of modern man. Here the resemblance is due to different factors working; in the case of round-headed man to the decrease in muscles and to upright stature; in the case of the apes the occiput is flattened to provide attachment for strong muscles reaching up from the neck region to support the head. Man's upright posture obviates the need for such marked occipital support, and the posterior projection of the occipital accounts largely for his greater length of head as compared with that of the apes. But this is only to say again that mere resemblances do not count for much; they must be interpreted in the light of the causes which give rise to them.

A word as to the bearing of these findings upon theories of evolution. If they look Lamarckian, that is only because the reader has not penetrated beneath the surface of the argument. This is not the place to discuss the relative merits of Darwinism, Weismannism or Lamarckianism, but if we understand their respective implications, there is nothing in our view which would not fit into any one of those schemes. The modification of form through function can proceed from generation to generation by the principles of Darwinian selection, if that is the evolution doctrine to which one is committed. It can proceed, of course, with the mechanism represented by Weismann. Likewise, it is susceptible of Lamarckian interpretation if one be a Lamarckian.

But in any case we can not afford to blind ourselves to facts because we may shy from their implications. A good case is not strengthened by adducing poor reasons in support of it, and no fear of giving comfort to the enemy should lead us to suppose that a partial concealment of truth, which arises from concealment of part of the truth, can compensate for the value of unprejudiced consideration of the facts of life, whether they seem to fit into our larger scheme of evolution or fail to fit. No one can read the history of physical anthropology since the day of Darwin without seeing that the evolutionary idea has largely dominated its ambitions and determined its findings, sometimes, we are convinced, to the detriment of the truth. The duty of the anatomist, however, is not to plead a cause but to play judicial advocate, willing to hear and consider all evidence bearing on the case.

Our findings mean, possibly, nothing more than that the human and the simian type, respectively, have been relatively fixed for a much longer period than it has been our custom to regard them. We are constantly lengthening out the vistas of the past, and it may be that we must extend them beyond our present wont in order to find the point where human and simian forms have diverged into their present persistent types. Certainly one can no longer accept Java man as common ancestor, nor do any of the Tertiary remains of simia suggest common ancestry. If it be clear that present evidence is insufficient, that fact provides sufficient answer to the question of how we ought to interpret such evidence as we have. We must not convict the prisoner at the bar simply because we do not know who else committed the crime. The essential point is, Can we prove him guilty? And so with regard to a common ancestor.

TROPICAL CYCLONES AND THE DISPERSAL OF LIFE FROM ISLAND TO ISLAND IN THE PACIFIC

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EVIDENCE FOR AND AGAINST A PACIFIC CONTINENT

ONE of the mooted questions concerning the Pacific is how the biota now found upon the scattered islands got there. There are two great schools of thought in respect to the matter. One believes that the presence of numerous Asiatic forms can only be explained on the assumption that at some earlier time many of the now remote islands were connected into a continent. Some members of this school think that the continent extended as far as the Low Archipelago and Hawaii.[1] Others do not ask for so much land, but assume a southeastward extension of Asia, to include the East Indies, Philippines, New Caledonia, Fiji and Samoa. Certain large islands are also believed to have existed, for example, one that would include all the Hawaiian Islands and another that would unite the Cook, Austral, Society and Tuamotu islands.[2]

The other school of thought is opposed to the idea of a Pacific continent, or of vast islands in it, and to the extension of Asia and Australia beyond New Caledonia.[3] They contend that the agencies distributing life from island to island are sufficiently efficient to have enabled the land forms to spread to the remote islands upon which they are found.

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[1] Campbell, Douglass H., "Some botanical and environmental aspects of Hawaii," *Ecology*, Vol. I, pp. 257–269, 1920; Bryan, Wm. A., "Hawaiian fauna and flora," Proceedings First Pan-Pacific Scientific Conference, Vol. 1, p. 158, Honolulu, 1921; Scharff, R. F., "Distribution and Origin of Life in America." 1911.

[2] Pilsbry, H. A., "The dispersal and affinities of Polynesian land snails," Proceed. Pan-Pacific Scientific Conf., Vol. I, pp. 147–151, 1921.

[3] Many geologists and biologists belong to this school, including T. C. Chamberlin, C. Schuchert, H. E. Gregory, D. S. Jordan, F. Muir, etc.

However, there are several features of the distribution of land forms that are difficult to explain on the basis of prevailing winds and ocean currents. One is the fact that many of the forms of even the easternmost islands are related to Asiatic forms rather than to American forms. That is not true of all types of life; a considerable number of Hawaiian forms, for example, being derived from a few American ancestors. Many of the seed plants, birds and some insects were derived from Central American ancestors.[4] But a large enough percentage of the forms are so Asiatic as to have led those scientists who have examined this particular type of evidence to doubt if the existing distribution can be explained on the basis of existing conditions. They have shown, for example, that a favorable wind (the prevailing trades) and current (the drift set in motion by the trades) should have given Hawaii many Californian and Mexican forms of littoral mollusca, if these agencies were effective in transporting these forms.[5] Likewise, the believers in the theory of widespread lands and certain other workers point out that the prevailing winds and currents throughout the Pacific tropics are westward, and the numerous species of Asiatic descent must have advanced eastward against the prevailing winds and currents. Yet the wind and currents are the only important agencies, aside from man, that might have transported the plants and animals in question from island to island.

How Hurricanes Can Distribute Life

Is it not highly probable that tropical cyclones have played a part in the dispersal of life from island to island in the Pacific? Upon their equatorward side there are often violent westerly winds, completely overcoming the prevailing easterlies. As pointed out in discussions of the courses followed, many storms move eastward, within the tropics, or just beyond the tropics.[6] In moving eastward, the strong westerly wind on its equatorward side carries much with it, and sets up a strong drift as well. An illustration of the occasional power and persistence of this westerly wind is given by the renowned missionary John Williams, who, driven by cannibals from Hervey Island, drifted in an open boat five hundred miles to Tahiti with a constant westerly wind.[7] Normally, easterlies prevail in that portion of the ocean.

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[4] Brown, F. B. H., "Origin of Hawaiian flora," Proceedings First Pan-Pacific Scientific Conference, Vol. 1, pp. 131–142, 1920 (seed plants); Muir, F., "Some problems in Hawaiian entomology" (insects), *ibid.*, and Henshaw, "Fauna Hawaiiensis," Volume Vertebrates (birds).

[5] Muir, F., *loc. cit.*

The power of the wind to transport light objects through the air is frequently illustrated during hurricanes, as, for example, when land birds and insects are carried out to sea in large numbers. Indeed, the presence of butterflies and birds far out at sea has often been mentioned in connection with hurricanes. A specific case of interest is mentioned by Dr. F. Woods-Jones[8] concerning insects at Cocos-Keeling Island in the Indian Ocean. During a severe northwesterly wind associated with a hurricane, hundreds of dragonflies were driven the seven hundred miles from Sumatra and Java, the nearest land, to this little island. However, as the island possessed no suitable environment for dragonflies the introduction was ineffectual in spite of the large numbers driven there. But if a marsh or other favorable environment for these insects should develop on the island, there is no question that within a short time some storm wind would stock it with dragonflies.

An actual increase of the permanent fauna of Cocos-Keeling Island took place during Dr. Woods-Jones' residence there, shortly after they had added a new plant, tomatoes grown from seed. At the first the tomatoes had no enemies, but before the first crop was ripened, an insect pest was brought by the same cyclonic gale that brought the dragonflies from Java and Sumatra. Although Cocos-Keeling Island is in the belt of Trades, which blow with exceptional strength and persistence from Australia, almost no Australian species are found in the fauna. Dr. Woods-Jones believes this fact is an illustration of Alfred Russell Wallace's generalization that flying forms are rather well adjusted to the prevailing winds, just as aquatic forms living in a regular current commonly are adjusted to it so as not to be swept away. But the sudden, irregular, violent winds and currents sometimes associated with tropical cyclones take many individuals by surprise and carry them away from land.

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[6] Visher, S. S., "Tropical cyclones of the Pacific with charts of tracks," *Monthly Weather Review*, Vol. 50, pp. 288–297, 583–589, 1922, and bulletin of Bishop Museum of Honolulu, 1924.

[7] Williams, John, "Missionary Enterprises in the South Sea Islands," London, 1838.

[8] Professor at Adelaide University, Australia. Remarks following my paper before the Royal Society of New South Wales, November, 1921.

Not only are animals with wings sometimes carried long distances by hurricane winds, but many forms attached to leaves are thus carried. The stripping off of almost all the leaves occasionally accomplished by hurricanes has been mentioned often. Some of these leaves are carried far, and on these parachutes are sometimes attached worms, larvae eggs or small snails.[9]

HURRICANE CURRENTS AS DISPERSING AGENCIES

The strength of the abnormal ocean currents set in motion by hurricane winds is mentioned repeatedly in the Pacific Islands Pilot.[10] The significance of such abnormal currents may be illustrated by a statement concerning the Hawaiian Islands. A few years ago the mangrove was introduced upon the island of Molokai. In spite of strong normal currents at right angles to the favorable direction, seeds are occasionally floated to Oahu by abnormal currents, and the tree is now establishing itself there, more than twenty-five miles from Molokai.[11]

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[9] Pilsbry, H. A., "The dispersal and affinities of Polynesian land snail faunas," Proceed. First Pan-Pacific Scientific Conference, Vol. 1, p. 149, Honolulu, 1921.

[10] U. S. Hydrographic Office, Washington, 1920.

The facts that most of the lowland plants and animals of the wide stretch from Fiji and the Carolines to the Low Archipelago and Hawaii are of the same or closely allied species, and that at the same time there is a progressive dropping out of species eastward, suggest strongly that the distribution of life forms has been from scattered island to island and has been accomplished by transporting agencies that are rather efficient, though not perfectly so. The fact that relatively few forms are of American origin (except remotely) suggests the inadequacy of the normal trade winds and normal currents as agencies of dispersal, in accord with Wallace's law concerning normal winds and currents. However, the relatively small part that South America has played in supplying forms to the Pacific Islands doubtless is due partly to two special conditions. One is found in the few islands in the eastern part of the tropical Pacific. Indeed, good atlases show no islands between the Low Archipelago and South America, about three thousand miles away. The Galapagos Islands, on the equator, are almost the only islands north of that zone in similar longitudes. South of 24° S latitude are only Easter Island (28° S), Sala Y Gomez (26½° S), and a few other islets or rocks to the west and northwest of Easter Island, and also San Felix and Juan Fernandez islands near the 80th meridian, not very far from South America.

The second special condition highly unfavorable to the spread of South American forms over the Pacific Islands is the fact that much of the western portion of tropical South America is almost barren of life, on account of the extreme aridity of the lowlands and the presence of the lofty Andes only a short distance from the coast. Somewhat similar conditions obtain over a wide belt in the North Pacific. There are almost no islands between Hawaii and North America, and most of the coast of Mexico is almost barren on account of aridity. On the other hand, the southeastern coast of Asia and the East Indies teems with forms adapted to the climatic and soil conditions which obtain in most of the Pacific Islands.

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[11] Oral communication by C. S. Judd, territorial forester. Several illustrations of the significance of changes in ocean currents in the Atlantic are given in papers in the Proceedings of the First Pan-Pacific Scientific Conference, Vols. 1 and 2.

Another argument of advocates of great extensions of the lands is the following: they say that wind and currents, even those associated with hurricanes, apparently are not effective in the dispersal of certain types of life, as shown by the absence from the coast of Queensland of certain forms known in New Caledonia. Both the normal trades and numerous hurricanes pass over New Caledonia and thence to Queensland, and yet the coconut is established at only a few points on the Queensland coast, and certain species of land snails abundant in New Caledonia are unknown in Queensland.

The general lack of the coconut in Queensland appears not to be due, however, to a lack of seed there, for they are often noticed sprouting along the shore. The severe droughts during the cooler season of each year, characteristic of this part of Australia, may be the factor preventing their widespread establishment on this coast.

The absence from Queensland of the large showy land snail (Placostylus), common on the islands to the eastward, may be due to the presence in Australia of enemies that prevent their establishment. The Australian Bush-turkey feeds upon similar mollusca, and it has been suggested by Curator Charles Hedley, of the Australian Museum, as possibly responsible for the absence of this island snail in Queensland.

HURRICANE FLOODS AND LIFE DISPERSAL

Another way in which hurricanes have influenced the dispersal of land forms is in connection with the floods they cause, whenever excessive amounts of rain fall in a short time. There are numerous records of the fall of more than ten inches in two days, and some records of more than sixty inches in three days.[12] Under such conditions normally rather small streams become rivers, and carry to sea vast quantities of driftwood. The river banks are eroded badly, and many trees are undercut and carried out to sea. During the excessive rains, large masses of dirt and loose rock upon steep hillsides often slip or slump, sometimes temporarily damming valleys. In case the dam breaks, the sudden rush of waters does its part to contribute natural rafts of driftwood, with their load of land animals and seeds. Furthermore, the animals are not nearly so likely to be picked off a raft by sea gulls or other birds during a hurricane as they are in ordinary weather. Hence, the absence of long rivers flowing into the Pacific, with the exception of the Chinese rivers, should not lead to the assumption that natural rafts of considerable size and biological dispersing possibilities are lacking in the Pacific.[13]

Tropical cyclones have also played a part in the distribution of peoples over the Pacific. Legendary accounts indicate that a number of island groups were discovered by occupants of boats that had been blown out of their course by storms. The discovery of New Zealand by Polynesians enroute from Samoa to the Cook Islands is a specific illustration.[14]

MAN AS AN AGENCY OF DISPERSAL

The great importance of man in distributing plants and animals of economic importance and, incidentally and inadvertently, of numerous smaller species concealed in the soil or bark or on leaves has been emphasized by several scientists.[15]

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[12] Visher, S. S., "Tropical climates from an ecological viewpoint," *Ecology*, Vol. IV, pp. 1–10, 1923, and "Tropical cyclones of the Pacific," Bull. Bishop Museum, Honolulu, 1924.

[13] Pilsbry, H. A., (*loc. cit.*) has made this erroneous assumption. He states: "The argument for distribution of animals by natural rafts has never been more convincingly stated than by W. D. Matthew in his paper 'Climate and evolution.' Much of his argument, however, is not applicable to the Pacific Islands. Here we have no large rivers to give forth natural rafts. If a single tree is washed to sea it must be very exceptional."

[14] Best, Elston, "Polynesian navigators, their exploration and settlement of the Pacific." *Geographical Review*, Vol. 5, pp. 169–182, 1918.

GEOLOGIC CHANGES

The dispersal of life from island to island over the Pacific has been accomplished slowly. Undoubtedly, during the geologic ages, there have been geologic changes that have been significant. For example, it is known that long ago there was more than once a strait where the continuous Central America now is. Conceivably at such times the drift induced by the Trade winds, and which is now diverted northward in the Atlantic to form the Gulf Stream, may have continued westward, carrying with it some of the seed plants and animals of the islands situated where Central America now is.[16] Likewise, any tropical cyclones that occurred then in the Caribbean region would have been more likely than now to sweep westward far into the Pacific, for the atmospheric pressure conditions prevailing over the land, especially mountainous land, seem often to divert tropical cyclones.

Likewise, it is known that Australia was formerly connected with Asia by way of the East Indies and New Caledonia. At such times, it is probable that the normal ocean currents were distinctly different from the present. While now part of the equatorial current finds its way westward between the islands and enters the Indian Ocean, formerly the continuous land necessarily diverted the warm equatorial current into higher latitudes.

Another change during the geologic past, which is much more frequently mentioned, is the lowering of the sea level during the accumulation of the continental glaciers of the several ice ages. It is thought by some that the sea level was lowered about sixty feet during the Pleistocene glacial epochs. Unquestionably there was some lowering of the sea level, but probably not nearly that much, for the glaciers probably were not nearly so thick as some have assumed.[17] However, any considerable lowering of the sea would result in an increase in the land area, sometimes connecting neighboring islands, and hence facilitating the local spread of land forms.

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[15] For example, by Mayor, Alfred G., and Safford, W. E., in Proceedings First Pan-Pacific Scientific Conference, Vol. 1, pp. 15, 147, 183–187, and by Best, Elston, *loc. cit.* Safford has made especial study of the cultivated plants of Polynesia, *loc. cit.*, pp. 183–187. See also Guppy, H. B., "Observations of a Naturalist in the Pacific," Vol. 2, on "The distribution of plants and animals," London, 1908.

[16] Brown, F. B. H., *loc. cit.*

DURATION OF GEOLOGIC TIME

The recent great increases in the estimates of the duration of the geologic past also has a bearing upon the subject of tropical cyclones and the dispersal of life in the Pacific. It is conceded that the chances of a single hurricane doing much along this line are small. The great reason why hurricanes have been largely ignored by the students of this problem is because hurricanes were believed to occur only at long intervals and were thought to be too rare to play much of a part. But now that it is known that more than a score occur annually, on the average, out in the Pacific (in addition to those in the Philippine region) they take on a different aspect. Their significance is also increased by the fuller appreciation of the diverse ways in which they affect the lands, streams and currents. They are also less likely to be ignored as a factor in the dispersal of life when it is realized that the interval since the beginning of the Mesozoic Era probably is many times the twenty million to fifty million years it was thought to be a few decades ago. Indeed, the remarkably convincing evidence, derived by leading physical chemists from the study of the products of the disintegration of radioactive substances in igneous rocks of various geologic ages, indicates that probably more than a billion years have elapsed since life became abundant upon the earth. Hence, fortuitous happenings, such as the dispersal of living forms by hurricanes, have had a long time in which to succeed.

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[17] Huntington and Visher, "Climatic Changes, their Nature and Causes," New Haven, p. 125, 1922.

SHORTER ARTICLES AND DISCUSSION

THE COMMENT ON SEX IN PIGEONS CONTAINED IN GEROULD'S PAPER ON BUTTERFLIES

In a semi-detached position at the end of a paper recently published in *Genetics*[1]—a paper dealing particularly with "white wing color in yellow butterflies"—Gerould[2] has discussed at some length two points involved in the work of Whitman and of Riddle on sex in pigeons. His discussion of the work on pigeons apparently has only a tenuous connection with his own study, but Gerould clearly gives his readers the following impressions: (a) That he has made and presented an "analysis" of the published data of Whitman and Riddle on sex-control; (b) that the statements made by myself concerning sex or sex-control rest merely or mainly upon the data which he claims to have reviewed; (c) that his "analysis" indicates that our data on this subject are without special significance.

Gerould presents so small a part of the two kinds of data which he assumes to analyze and in other respects is, quite unconsciously no doubt, so unfair, and the data involved are so little available to most readers of the journal which carries his article, that it seems necessary to call attention to some of the errors of his statement. Limitations of space, however, will permit neither the complete quotation of, nor special comment upon, all his remarks.

One should first consider the results obtained with butterflies which Gerould thought applicable to our results with pigeons—results which led to his meager examination of parts of the work of Whitman[3] and of Riddle ([4, 5, 6]). It would seem that the utter dissimilarity of reproductive conditions in these two groups might have suggested caution in making any comparisons whatever—particularly since, in the work of Whitman and myself, the sex-tendencies of ova were referred to highly specific conditions of reproduction in pigeons. But it appears that in the "paired eggs of pigeons" Gerould hoped to find a "precocity of male-producing eggs comparable to the normal condition in Colias." He, and apparently all others interested, already knew (Gerould, p. 543), in the case of the earlier appearance of males from a brood (a large group) of eggs in Colias, "that these well-known facts are not due to an earlier laying of the male-producing eggs, but probably to a greater rapidity in the development of the male as compared with the female." "Precocity" of male-producing eggs in Colias therefore refers solely to a *more rapid development of the male than of the female embryos* of that group; and for any elucidation of this point one would suppose it necessary to look to *this same kind of fact* in pigeons. On this special and pertinent point was our published statement, in a paper which he does not cite[7]—possibly published too late for his inspection—that in pigeon embryos "we are unable to say, however, that a difference in 'developmental rate' is found for male and female" (p. 244). At no other time or place have we ever suggested that such a difference exists. *A priori*, one might have thought that more light would be shed upon the phenomenon of "more rapid embryonic development" of the male sex in Colias by comparing this with the "rate of embryonic development of the sexes" in pigeons, than by fixing the comparison on "the order of output of ovarian eggs" in pigeons!

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[1] The management of *Genetics* was unable to publish a reply in that journal. In order that the readers of the original criticism may have the facts herein presented the author has been obliged to ask for publication elsewhere.

[2] Gerould, J. H., "Inheritance of white wing color, a sex-limited (sex-controlled) variation in yellow Pierid butterflies." *Genetics*, 8: 495–551, Nov., 1923.

[3] Whitman, C. O., Posthumous works; edited by Oscar Riddle. Vol. 2. Carnegie Inst. of Wash., Publ. No. 257, 1919.

[4] Riddle, O., "The determination of sex and its experimental control," *Bull. Amer. Acad. Med.*, 15: 265–284, 1914.

It seems, moreover, that the appearance of a "predominance of males in the early season" from certain crosses of pigeons was also at first thought to be comparable, or in line with, the "precocity"—the more rapid embryonic development—of males from normal matings of Colias. The statement just made above obviously applies also to this doubly mismated comparison. It is remarkable only that such comparisons were attempted.

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[5] "Sex control and known correlations in pigeons," AMER. NAT., 50: 385–410, 1916.

[6] "Control of the sex ratio," *Jour. Washington Acad. Sci.*, 7: 319–356, 1917.

[7] "On the cause of twinning and abnormal development in birds," *Amer. Jour. Anat.*, 32: 199–252, Sept., 1923.

These, then, were the mistaken considerations which led Gerould to some inquiry—in the work of Whitman and of Riddle—concerning the "paired eggs of pigeons" (two eggs of clutch, the first—from most pure species—predominantly male-producing), and the predominant production of males in early season followed by predominant female-production later in the season (this from certain crosses, and after forced and crowded egg production). We may next consider the nature and completeness of his inquiry.

Gerould reproduces one particular table (summarizing the sex production records of three females of one particular cross) from the Whitman (3) studies (nothing was taken from either of the three papers cited from Riddle), and then says:

> In the cross *T. orientalis* ♀ x (*St.*) *alba* ♂ an excess of males is said to occur in the spring months, whereas later in the season, *e.g.*, after July 1, females predominate. The offspring of three mothers are cited to establish this point. It is apparently on such meager data as these that Riddle's sweeping conclusions regarding sex control are drawn.

The actual facts could only have been stated in words similar to the following: That the offspring of these "three mothers" were specifically noted by the authors as *not* demonstrating, but as *bearing* on this point; that generic crosses generally, specific crosses also (72 types of crosses—listed on p. 10 of that study—practically all have a bearing), and much breeding without any crossing at all recorded in that volume, are all also cited as bearing on the matter; that in any and all cases *season* is not alone concerned, since several other modifying conditions were already named and described—and season itself is probably only indirectly involved through its relation to variable periods of fertility; that no attempt is made in the chapter cited, nor in other chapters of that volume, to present Riddle's studies on, or knowledge of, these several factors (for the reason that Whitman had recognized and considered only a few of them, and it was essentially his work that was being presented in the volume from which Gerould took one table); and that, in the same chapter from which this table was taken, it is plainly stated that all the data there considered "are perhaps not wholly conclusive. They do accord, however, with the results found generally in the other crosses, and a consideration of this matter may be undertaken in connection with these data" (p. 72).

But that the evidence for "*sex control*" in these or any other particular cases should be asserted to rest solely or mainly upon even all the "sex ratios" obtained is a further error which could not be made by any one who had read even the single chapter of the Whitman volume containing the table cited by Gerould. For example, in connection with this topic—seasonal predominance of the sexes—the following statement (p. 71) was there made:

> The editor (*i.e.*, O. R.) is making extensive studies on many subjects connected with the size, chemistry and stored energy of the egg, and of the relation these bear to sex and fertility; on the relation of season and "crowding" of clutches to sex; and the question of the relative infertility of these hybrids. The results of these studies must appear later. The additional knowledge obtained from these sources, however, gives the editor confidence concerning some points of the present chapter which do not appear conclusive from the data available for the present volume.

Nor could this error be made by any one who had read and partially comprehended either of my own three short publications cited by our critic. One may therefore assume that Gerould had completely read none of the citations made to the studies of Whitman and of Riddle. In all my own three publications cited by Gerould there was plainly presented, by means of a figure or diagram, the *eight* (1914) or *ten* (1916, 1917) additional and distinct lines of study then being conducted, and which constitute my own *tests* of the reality of "sex control"; and all or several of these tests were also briefly described in the text of each of those three publications. Additional kinds of tests have been added and partially reported since 1919.

From the above it follows that in no sense is it true that "it is apparently on such meager data as these that Riddle's sweeping conclusions regarding sex control are drawn."

But Gerould further finds that the data of the single table actually examined are also unsatisfactory. In opposition it is stated: "The numbers are small and indicate a slight excess of male offspring." Surely the numbers (also the amounts and kinds of data examined!) are small. But what has the statement that the numbers "indicate a slight excess of male offspring" to do with the question? Is not the question whether these particular records do or do not show a predominance of males from the earlier season and a predominance of females later in the season? Could not the case be proved perfectly with "a slight excess of male offspring"? Or, equally well with a slight excess of female offspring? Or, with exactly equal numbers of the sexes? This fallacy is again belabored within his paragraph when it is stated that

> The small numbers of offspring (13 ♂ ♂, 16 ♀ ♀) of this mother (No. 54) indicate that the seasonal variation in the sex ratio is quite within the range of merely chance variation.

Despite the small numbers involved when single years are compared, and notwithstanding the amount of "lumping of data" which we warned was practised in such a summary, it can be seen by reference to the data for these individual birds (3; pp. 52, 53, 78, 79) that the sex ratio before and after July 1 is different in six cases (two cases showing equality include the *smallest* numbers of the series). In all these six cases the proportion of males is higher before July 1 than after. And, after separating the final period for one of these mothers—following November 29, 1914, and forming the actual beginning of a true early season period (as provisionally noted in table 44)—the total numbers obtained from the three mothers are 34 ♂ ♂ : 11 ♀ ♀ from the earlier period, and 26 ♂ ♂ : 32 ♀ ♀ from the later period. The two years (1906, 1909) of no "overwork" gave 5 ♂ ♂ : 1 ♀. Whoever wishes to do so may of course assure himself that these several figures are "quite within the range of merely chance variations."

Concerning the first of the two points discussed by Gerould it thus becomes clear (a) that his attempted comparisons between Colias and pigeons are unsound, (b) that he very inaccurately states the problem of periodicity in sex production and mentions no other factor than season as related to it, (c) that he ignores the fact that all the publications cited by him make it entirely clear that only a part of the data for any aspect of sex control has hitherto been published, (d) that he utilized only a part of the published data bearing on the specific question raised, (e) that he draws an unwarranted conclusion from the fraction of data which he examines.

Gerould next presents an "analysis" of a second point in the work of Whitman and of myself—the predominance, in some pure species, of males from first and of females from the second egg of the clutch. Here again we find: (a) He ignores completely—never once mentions—the point chiefly involved in a discussion of this point (in so far as my own work is concerned), namely, the relation between yolk-size and sex; (b) he has totally failed to grasp or to intimate the fact that our studies on "sex control" manage to get on whether the eggs come in "normal order," "reversed order," or utter "disorder"; (c) he utilizes a part only of the only kind of evidence to which he refers; (d) he presents statements from other workers on hybrid forms (Cuenot, Cole) as though they are in contradiction instead of in complete confirmation of any words ever used by Whitman or myself on the subject; (e) he again refuses to draw the sane and necessary conclusion from the data actually cited.

A closer examination of all these several points can not be included here. Perhaps it will suffice to refer the reader particularly to pages 48, 72, 172, 173 of the Whitman (3) studies; to note that, in addition to the papers cited by Gerould, the writer has published twenty-two papers which deal with one or another aspect of the problem of sex control and sex production in pigeons; and to supply the accompanying tabulation of data on "sex predominance in the two eggs of clutch" contained in the particular publications which Gerould cites and presumably reviews.

The reader may next examine the summary table just mentioned. This table further indicates (by means of figures in parentheses) the part of these data actually found and utilized by our reviewer. Among hybrids only a single kind (one or another race of common pigeons) is cited by us here, though many kinds are available. Fragmentary data (or data having no relation to other problems) on common pigeons were not included in the Whitman publication; and, in the papers cited by Gerould, the present writer has published nothing whatever on this group. A few data for *C. livia* and *C. affinis* (wild) were not included here because breeding data have shown (3; p. 112) some of the individuals used were not pure but hybrid; none of my own data on these species is published. Individuals of *St. alba* whose purity was questioned for other reasons are also excluded. Otherwise, all data for all groups contained in the four publications cited by Gerould are included.[8]

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[8] The pages of the 1919 publication (3) on which the data may be found are as follows: No. 1; 34, 38, 39, 40, 51, 52. No. 2; 35, 38, 139, 140. No. 3; 117, 118. No. 4; 119, 120, 121. No. 5; 167, 168, 169. No. 6; 171. No. 7; 171. No. 8; 150, 153. No. 9; 81, 85, 96, 140, 145, 148, 151, 157, 164, 174, 175, 177 (Riddle, 1916: 392, 393). No. 10; 76, 77, 83, 84, 140, 145, 157, 163. No. 11; 148, 149. No. 12; 93, 104 to 110.

The table shows with sufficient clearness that only a part of the data in the studies cited was actually considered by our critic. But even the part considered he finds quite unsatisfactory. Why? He refers to six species. In every one of these six species the "sex pairs" did yield, in plain fact, more males from first eggs and more females from second eggs; and this is the sort of "predominance" of which we have repeatedly spoken—with the additional statement that "it seems probable that this order is normally reversed in some other wild species. . . and also in some individuals, etc." (3; p. 173). Again, the

TABLE 1

Data hitherto *published* (summarized here for first time) on order of males and females in pairs of eggs which produced the two sexes. Group 1: Pure wild species. Group 2: *Streptopelia* species, individuals of which are certainly sometimes contaminated with a related species. Group 3: Various races of common pigeons—*all hybrids.*

Group	No.	Kind of pigeon	Number of cases of:			
			Males from first egg and females from second egg of pair		The reverse	
	1	Turtur orientalis	23	(10)[9]	9	(2)[9]
	2	Turtur turtur	4		2	
	3	Zenaidura carolinensis	5		2	
1	4	Zenaida vinaceo-rufa	5		0	
	5	Spilopelia suratensis	5	(4)	3	(3)
	6	Phaps chalcoptera	4	(4)	0	(0)
	7	Stigmatopelia senegalensis	2[10]	(2)[10]	0	(0)
	8	Streptopelia humilis	4		1	
			—		—	
		Total	52	(20)[9]	17	(5)[9]
	9	Streptopelia risoria	38	(21)	20	(8)
2	10	" alba	13	(7)	6	(5)
	11	" douraca	1		2	
		Total	52	(28)[9]	28	(13)[9]
			—		—	
3	12	Common pigeons (homer; pouter; domestic rock)	10		9	

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[9] Cases cited by Gerould completely given in these columns.

[10] 12 ♂, 2 ♀ from first eggs; 2 ♂, 9 ♀ from second eggs (unpaired hatches).

small numbers of "unpaired" (sex known from one egg only) individuals cited do positively indicate the same thing. If, in disregard of this uniformity of the *six* groups, he was unwilling to conclude that the observed differences are significant the additional data available to him already prove his estimate a poor one. Such additional data were available for four of those six species, and in every case the earlier type of difference is clearly maintained when the additional or missing data are found and tabulated. Moreover, four additional (unmentioned by our reviewer) species of unquestioned purity all reinforce the evidence from the other six. All these ten species show—*despite the small numbers* obtained for most species—a predominance of males from first and a predominance of females from second eggs. All individuals of eight of these ten species were unquestionably pure, not hybrid. The total numbers for this group are, 52 to 17—a little better than a 3:1 ratio. Only one of the 11 species (*St. douraca*) represented in the table seems not to present a similar situation and here the numbers are the smallest of the series; in addition, the ancestry of the bird which produced the two reversed pairs (3; p. 148) was not recorded and may not have been pure *douraca.* Further, we have stated no "rule" for *all* pure species. For the three species of this second group, which almost certainly includes some *individuals* which were not pure, the numbers obtained are 52 to 28. Finally, the only hybrids listed (homer, pouter, etc.) show essential equivalence, 10 to 9.

Perhaps these paragraphs will assist the reader to a truer estimate of the nature and extent of Gerould's "analysis," and of the soundness of the conclusions which he draws from it.

In conclusion, I trust it is wholly true that I have made some "extensive conclusions regarding sex control." I am now finding occasion further to extend some of them (and to qualify a few). Any statements made, however, have been made upon the basis not only of all my own data—published and unpublished—and of those of Whitman, but upon all other relevant data known to me as well. Fortunately, the present attainment of analogous sex studies in the hands of others is such that the essential points in physiological sex theory urged by Whitman and by myself would now be largely established even if our work be left quite out of account. That advance is now well under way—so well, that one may even now recall the exhilaration of falling into step when the line was thin and the goal in doubt.

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THE EFFECT OF X-RAYS ON THE FERTILITY OF RATS

THIS paper is a preliminary report of some work on the effects of X-rays on rats, conducted in the genetics laboratory of the Bussey Institution. The X-raying itself was done at the Jefferson Physical Laboratory, and acknowledgment is due to Professor William Duane and Dr. G. L. Clark for generous assistance in devising apparatus and help in using it.

METHODS

Only males have been rayed, and only the region of the testes themselves has received the rays. To accomplish this, a wooden box was completely sheathed with sheet lead 2 mm in thickness. To the inner side of the sliding door of the box the rat to be rayed was fastened. When the door is slid into position, the scrotum of the rat comes exactly opposite an oval opening in the lead sheathing. Thus the whole body of the rat, except the region of the scrotum and the parts immediately overlying it, will be protected from the rays, the object being to ray the germ-cells and as little besides as is possible. See Figures 1 and 2.

In order to be positive that rays entered the box, a photographic plate was wrapped in black paper, and placed in the box in the same position as the rat would be placed. It was exposed for three seconds. The result was that the plate was blackened strongly and uniformly over the area opposite the opening in the lead sheathing, but nowhere else.

The rats were etherized, to keep them quiet, strapped to the door, and exposed to the X-rays for varying lengths of time. On dosages of more than 20 minutes, the rat was rayed in twenty-minute stretches, to insure his remaining quiet and in position throughout the treatment, with 20 minutes between successive exposures. A convenient voltage and amperage were arbitrarily selected, as follows: 65,000 volts, 3 miliamperes. The rats were placed at a distance of 40 cm from the edge of the bulb. A tung-

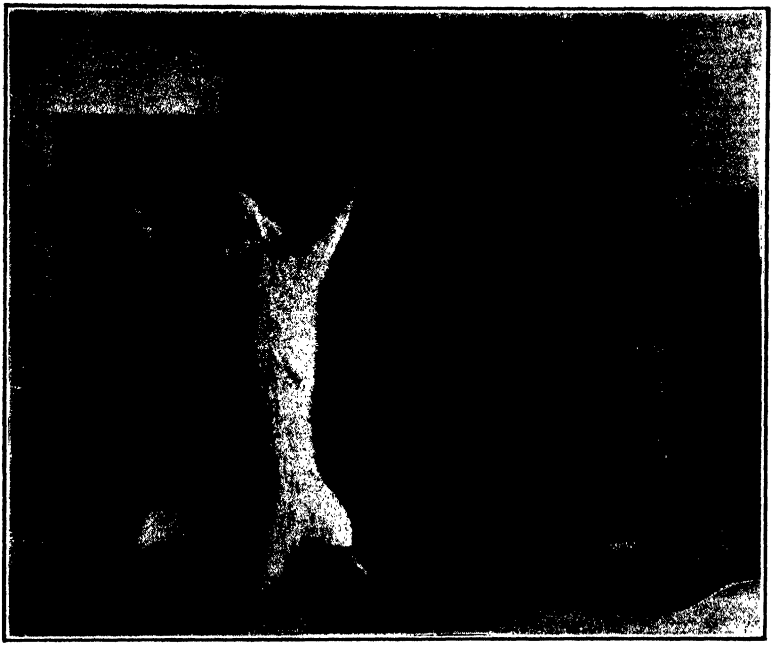

FIG. 1. X-ray box, front view, showing oval opening in lead sheathing and rat strapped to sliding door. Lead cover for the box is shown at right.

sten X-ray tube was used. These relations were kept constant throughout the experiments, but the time of exposure was varied.

At first a thick aluminum filter was used: later a thinner one. Then, as no "burning" effects were found, the filter was discontinued.

The experiment was started by exposing rats for periods varying by five-minute steps from one minute to an hour. Each X-rayed male in this experiment was tested for fertility by keeping it constantly associated with fertile females, alternating it every three weeks between two cages of females, so as to further insure a valid test of its fertility.

RESULTS

It was found that all rats rayed up to an hour, using the thick filter, remained perfectly fertile, regularly producing normal, healthy litters. Then a thin filter was used. Rats rayed up to 35 minutes were normally fertile, but those rayed for 40 minutes

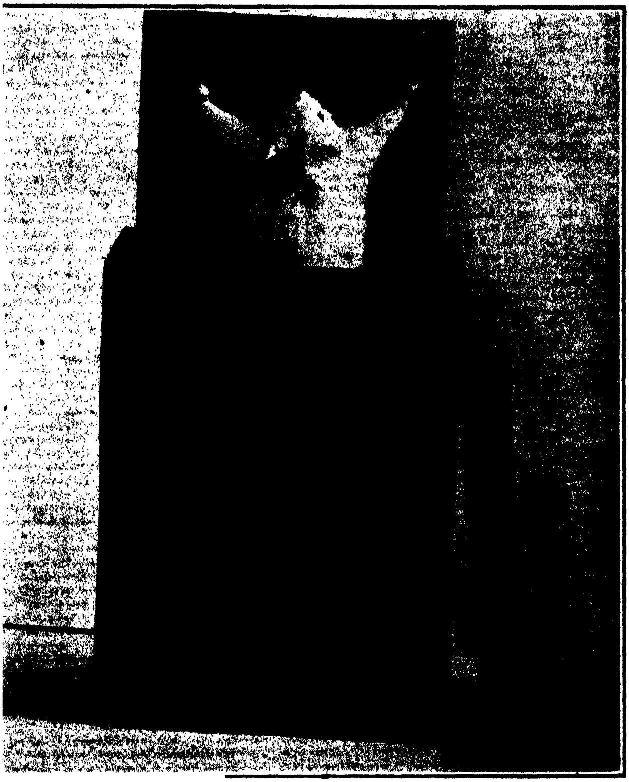

FIG. 2. X-ray box, back view, showing rat being placed in position.

went through a sterility period, to be described farther on. When this result was found, the filter was discarded altogether; and rats rayed without the filter behaved just as rats rayed with the thin filter, that is, exposure up to 35 minutes did not effect fertility, while a 40 minute exposure caused the sterility period. It should be noted, however, that with the thick filter no sterility followed, which indicates that the soft rays, of long wavelength, are the effective rays.

The rats which were rayed for 40 minutes with a thin filter or with no filter, behaved as follows. For the first few weeks they were normally fertile, litters being produced as long as 66 days after raying. After this fertility period, which varied in duration among the individual rats, a sterility period, quite marked, appeared, during which absolutely no young were produced.

After two months or more of sterility, fertility again appeared in full force, large healthy litters being produced regularly. Table I shows the details of the sterility periods to date.

TABLE 1

Rat No.	Litters produced after x-raying and before sterility.	Average young per litter.	Time from x-raying until last litter produced before sterility.	Time from x-raying until first litter produced after sterility.	Sterile interval.
10	6	6	55 days	120 days	65 days
66	6	5	66 days	110 days	44 days
167	3	6	62 days	146 days	84 days
182	*	*	*	136 days	*
185	1	5	24 days	156 days	132 days
215	3	6	65 days	119 days	54 days
227	2	8	27 days	150 days	123 days
231	3	5	49 days	141 days	92 days
average			49.7 days	136 days	85 days

The litters produced before sterility are presumably produced from sperm mature at the time of X-raying. If this is so, we may have a clew to the length of time mature sperm can live in the testis and epididymis. The first litter produced after sterility may well be considered the critical litter, as it is produced from germ-cells which must have just escaped being killed. These might conceivably be affected in some way. Therefore the first litter produced after sterility in each case is being kept and carefully studied.

To date, eight litters have been recorded, each of which is the first litter produced by some male after his sterility period. From some of these, F_2 young have been raised, all of which appear normal. These are being back-crossed to their parents, as a means of detecting any recessive genetic changes which may have occurred.

Although as yet no genetic changes due to the X-rays have been detected, the fact is thought to be of interest that a differential resistance apparently exists between spermatozoa and some early stage of the germ-cells on one hand, and the intervening develop-

* Kept from females until 75th day.

mental stages on the other, the latter obviously being much more sensitive to treatment. An effort will be made to determine the sensitive stages more precisely.

POSTSCRIPT

Not until this paper was ready for press did I discover a preliminary report by D. R. Hooker, in the *American Journal of Hygiene,* II, Proc. 461–2, on the effects of X-rays upon rats. Apparently, however, Hooker's approach to the problem is from a medical standpoint rather than a purely genetical standpoint, and I do not think that my work involves any unnecessary repetition of Hooker's experiments.

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EVIDENCE OF STRUCTURE IN THE GENE

IN the last few years the amount and variety of data concerning gene variation have reached a point where it should be possible to make a general explanation that will at least serve as a working hypothesis. An explanation of gene mutation is suggested by some recent results from the highly mutable allelomorphs of the bar series in Drosophila. These results indicate that the gene consists of a main particle, or base, firmly fixed in the chromosome, with varying numbers of other particles, or modifiers, attached to it. The modifiers of any gene are usually attached to the base in a side-chain arrangement. Mutation consists of the gain or loss of modifiers or chains of modifiers.

(1) Two cases of coincidence of mutation occurred where one full-eyed mutant and one ultrabar-eyed mutant were found among the offspring of a single bar female. The female is emphasized since other evidence indicates that almost all the mutations of the bar gene occur in the female. The male in one case was bar-eyed and in the other full-eyed. The probability that these mutants were due to independent causes is of the order of one chance in a million. Bar eye is intermediate between full eye and ultrabar eye. In these cases both extremes arose simultaneously and it seems that one of the two bar genes in a germ cell of the female had lost something and that the other gene had gained something.

(2) The facet count of homozygous bar females is strikingly similar to that of full-ultrabar heterozygotes in those cases where the three allelomorphs of the series are derived by mutation from one stock and hence have the same titre of accessory factors. One dose of ultrabar is as effective as two doses of bar in reducing the facet number of the full eye. The difference between the full gene and the bar gene is the same as the difference between the bar gene and the ultrabar gene.

(3) The evidence indicates that one of the two bar genes of a female germ cell had lost something and that the other bar gene had gained by exactly that same amount. On the general thesis that losses can occur more readily than gains, the relative rates of mutation in the two directions should show which had lost and which had gained. Zeleny's data show that bar mutates to full about twenty times as often as it mutates to ultrabar. Therefore, the full gene is a bar gene minus something and the ultrabar gene is a bar gene plus something.

(4) This something which the bar gene may gain or lose is the *modifier* and the part to which it attaches is the *base*.

Bar mutates to ultrabar about three times in a hundred thousand flies, presumably due to the addition of a modifier subtracted from the homologous gene in a female. The full gene, however, does not add a modifier to form a bar gene so readily. Among all the millions of fruit flies so carefully examined, only one bar mutant has been found. This indicates a different order of mutation. It seems most probable that the full gene consists of a base alone and that the bar mutant arose by the addition of a modifier from some other locus.

(5) The ultrabar gene with its two modifiers mutates to full with about the same frequency as the bar gene with its one modifier. This would not be expected if both the modifiers of the ultrabar gene were attached to the base, since two breaks would have to be made as against one in the bar gene in order to attain the full condition. Therefore, it seems that the second modifier of the ultrabar gene is attached to the first modifier and not to the base. Furthermore, ultrabar mutates to full almost twice as often as it mutates to bar. This is to be expected, since pressure applied to the chain of modifiers would cause the greater strain next to the base.

(6) Sturtevant and Morgan have demonstrated that bar to full mutation is accompanied by crossing-over in that region of

the chromosome. Crossing-over is generally thought to involve intimate contact at the point of breaking. The mechanical dislocation of the modifier of the bar gene due to this intimate contact can readily be visualized.

(7) Since the facet counts of full, bar and ultrabar males resemble the facet counts of the corresponding homozygous females more than any of the heterozygotes, it is evident that the ratio of the number of modifiers to the number of bases is the potent factor determining facet number. The rate of change of facet number caused by successive additions of modifiers to the bases should give some indication of the facet number of possible allelomorphs with more modifiers than ultrabar. Excluding full since it has no modifiers, and taking the average of the counts for the bar female and the full-ultrabar heterozygote, since they have the same modifier-base ratio; the four following numbers remain in Zeleny's series: 400, 35.8, 25.7 and 22.0. The successive additions of modifiers not only cause a decrease in facet number, but the rate of decrease and the rate of the rate of decrease also become smaller. This indicates that all further additions of modifiers will give a facet count very slightly smaller than ultrabar. In effect, these allelomorphs with four or more modifiers would be completely dominant over full.

(8) A number of other measured character changes caused by mutation at the bar locus agree with the facet number data in that bar is intermediate between full and ultrabar, and that the bar female is similar to the full-ultrabar female.

(9) This explanation of the phenomena of mutation in the bar series also applies to those cases in other animals and plants in which considerable data have been accumulated. In some of these series of allelomorphs, however, there are members that can not be construed as quantitative variations of one kind of modifier, and occasionally these qualitatively different members also show a marked change in the rate of mutation. In such cases it seems that more than one kind of modifier is acting.

(10) An inspection of the chromosome maps of Drosophila shows that the loci of the various mutants are not distributed at random throughout the length of the chromosome, but are concentrated into certain regions. A comparison of these maps with the cytological figures shows that in almost every case these highly mutable regions correspond with the points of spindle fiber attachment. If these maps give a true picture of distance,

it is difficult to see why this relationship should exist unless mutation involves a mechanical alteration of the gene.

A later paper will discuss the general application of this hypothesis more fully.

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ON THE OCCURRENCE OF THE LARVAL STAGES OF SCYPHOZOA IN THE ELKHORN SLOUGH, MONTEREY BAY, CALIFORNIA

To the writer's knowledge, the literature of scyphozoan development is conspicuously lacking in any account of the abundance and conditions of occurrence of the larval stages in nature. It appears that the accounts of the development of *Aurelia* are all based upon stages reared from eggs in the laboratory, or upon material procured from collectors at Naples. The only statements bearing upon the present data are the following, in Sumner, Osburn and Cole's "Biological Survey of the Waters of Woods Hole and Vicinity":[1]

> Young stages recorded by several observers as early as March; adults as late as September. Dr. Hargitt thinks it likely that sexual reproduction occurs in summer, the resulting scyphistoma larvae remaining in that condition till the following spring, when ephyrae are produced.

This paper, however, notes nothing further, either as to the abundance of the larvae or as to their habitat. Mr. G. M. Gray, of Woods Hole, in reply to an inquiry regarding these points, was unable to give the writer any definite information. Since any abundance at all comparable with that to be reported here could not have failed to impress the workers referred to above, it is believed that the present record may be of particular interest.

The accompanying map will suffice to show that the Elkhorn Slough constitutes the greater part of a considerable system of tidal estuaries emptying into the central part of Monterey Bay. The region of particular interest for this report (enclosed within a circle on the map) lies a few yards up the channel of a small branch (Bennett Slough) which joins the main channel at a point about one half mile below its mouth. This region may be conveniently located by the fact that it underlies a small trestle, over which the Pajaro Valley Consolidated Railway crosses Bennett Slough. On extreme low tides, the water nowhere in this region exceeds three feet in depth, and is considerably shallower over the greater part of the area in question. Here, along the sides of the channel, and over its bottom, a stratum of rather large rocks has been superimposed upon the sand and mud, presumably having been put there to support the railroad embankment.

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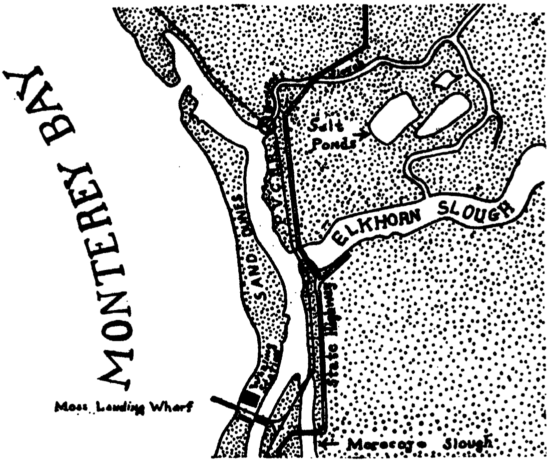
[1] Bull. Bureau of Fisheries, XXXI, 1911.



THE ELKHORN SLOUGH, MONTEREY BAY

In the early part of November, 1922, the writer discovered vast numbers of scyphistomae on the under sides of rocks in three small, localized parts of this region: (1) Well toward the bottom, a few yards below the bridge, on the south side of the channel. (2) in the middle of the channel, a few yards below the bridge, and (3) in very shallow water (1 foot at low tide), in the middle of the channel, a few yards above the bridge. In these spots the scyphistomae occurred in such dense profusion as to form numerous white patches, some of over a square foot in area, on the rocks. Their distribution is sharply limited to the above described regions; careful search for them on the piles and cement walls of the trestle, and of a near-by bed of eel-grass, having been absolutely fruitless.

Since the date of discovery, this association has been carefully observed at intervals covering approximately sixteen months. In all, thirteen trips were made to the locality. The results of these observations may be summarized as follows:

(1) Only scyphistomae were found in November and December, 1922.

(2) In February, 1923, most of the larvae had formed large, well-developed strobilae, and were actively giving off ephyrulae. The strobilae are of a light, brownish color.

(3) In April, 1923, many strobilae were still present. Many were quite small, and the general color was rather lighter than noted in February.

(4) On October 9, 1923, the same situation obtained as in November, 1922; only scyphistomae were found.

(5) Strobilae were not again found until March 14, 1924, although observations were made later in October, and in December, 1923, and on January 9 and 31, 1924.

(6) On each trip mentioned, larvae were found in the locations described in a former paragraph, but never elsewhere.

Unfortunately, it has, as yet, been impossible to determine definitely the species to which these larvae belong. All three stages seem to agree perfectly with the descriptions given for *Aurelia aurita.* However, it is possible that they may be the larvae of a species of *Pelagia,* or of *Chrysaora gilberti,* since adults of these species, as well as of *Aurelia,* have been observed drifting through the channel in the strong tidal currents. No analysis of the water of the region has been undertaken, though the nature of its fauna, consisting of numerous Hydroids, Chitons, Nudibranchs and Serpulae, and occasional starfish and actinians, would indicate a salinity not greatly different from that of the bay.

The presence of great numbers of larvae at all seasons recorded, and their absolute restriction to three very small areas would indicate to the writer that the aggregations of larvae persist for several years, under favorable conditions, and that after periods of strobilation occurring in later winter or early spring, the larvae remain quiescent as scyphistomae over the summer and fall months, again resuming the production of ephyrulae late in the next winter. At present it is, of course, impossible to make any statements as to the number of times this cycle may be repeated, or of the factors calling forth its various phases. However, if we suppose the foregoing account to be a correct one, this unique sequence of events may constitute favorable material for physiological investigations of the life history in general.

Both the extreme abundance of larvae in the region considered in this report and the evidence of the long span of their active life are suggestive of the type of resources which may account for the hordes of jellyfish which appear annually along our shores.

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SOMA AND GERM[1]

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THE conception that germ-cells are derived directly from the fertilized ovum and not from the tissues of the body and that they persist as distinctive elements side by side with the body material, dates apparently from the studies of Nussbaum on Rana, Triton and Salamandra, published in 1880. The tremendous impetus given by Weismann to this idea of the uniqueness and independence of the germ, in his challenge to the doctrine of inheritance of somatic acquirements, has made it the prevailing view to-day, and in the form of the familiar theory of germinal continuity it constitutes the very warp and woof of modern genetics. Since the time of its inception one important discovery after another in the fields of embryology, cytology and genetics has helped to build up the imposing body of facts which in the main substantiates it.

No biologist to-day, in the light of available evidence, would question the conception that the materials of the germ-cells have been derived, in the main at least, from the oosperm by a process of cell-division, for this is the obvious explanation of how hereditary traits already established are handed on.

Because a theory of germinal continuity accounts admirably for the transmission of established genes, however, it does not follow necessarily that it also accounts adequately for the acquisition of new genes or for the modification or loss of old ones. Manifestly the cash underlying a bank account can be transmitted from bank to bank indefinitely, but it takes some outside intervention to credit us with two dollars on the ledger where but one has stood before. In the end, in any such hand-and-glove relationship as exists between organism and environment, it would seem that directly or indirectly environment must play an important part in initiating, or at least in conditioning, changes.

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[1] Address of the retiring president of the American Society of Zoologists, Washington, D. C., December 31, 1924.

We may, perhaps, with some justification, look upon anything that environment brings out as in a sense hereditary—and one of our greatest biologists has apparently taken this stand—but if we accept this broad interpretation as the unqualified truth, then any argument about inheritance of somatic acquirements is pointless, since the very fact that it can be called forth or "acquired" through some specific influence of the environment shows that the "makings" were already present in the germ-plasm and that it only required a special environmental condition to call the character forth. In other words, the thing to be accomplished is already accomplished before it *is* accomplished. A very enigmatical situation, to say the least, I think you will agree. On this basis, then, unless biologists are unique creatures who dwell habitually in a topsy-turvy land, there must be two kinds of heredity; for when we demand evidence of the *inheritance* of such an acquirement we say it must not only be capable of being brought out in this unusual way but that also it must so change the genes of the germ-cells that they will produce the character again in later generations in an environment lacking the original evocative peculiarity.

Perhaps this is a foolish demand; however, it is the one usually made for proof of inheritance of a somatic acquirement. I am not solving the riddle, merely voicing it. On the other hand, is not stating the problem of heredity this way on about the same shadowy basis as affirming that the potentia of the oceans, continents and topographical features of the world to-day were present in the original nebula which preceded our solar system or that man lived potentially in some primitive protozoa-like creature? While in a sense this may be true I do not believe that the most optimistic of geneticists would expect to find the genes of man's present manifold attributes in such primitive protoplasmic creatures.

What seems to have happened in the evolution of living things is that such originally indefinite "might-bes" have become the present "ares." In other words, of the many possible outcomes inherent in the very physical and chemical nature of the vague initial life-substance, some have been realized. Furthermore, in the organisms of to-day this realization has progressed from the realm of indefinite possibility to a more or less specific certainty through the origination in cells of representative packets which we call genes. Through the action of these, subject to the existing environment, the characteristics of present-day forms are achieved. In other words, no matter how devious the path of procedure or how tangled the skein of chemical relationships, there is a certain determinate correspondence which did not exist in the primitive ancestral protoplasm, between special elements in the germ and the characteristics of the body as they are expressed to-day.

If this is untrue, then either there is no problem of evolution or it is wholly different from what it is commonly conceived to be. If it is true, then the great key problem of evolution—that of the origin of inheritable variations—is this very one of how the genes which exist in germ-cells to-day have been gradually incorporated in the erstwhile primitive protoplasm. This accumulation, which most of us take for granted, has not been a mere hit-or-miss affair, moreover, but an orderly, sequential process. Each new acquisition has been possible largely because of some earlier acquisition. In an organism the past history of a part is an important factor in its present expression. Where once reigned possibilities which might be epigenetically realized, there now exists a new, more specifically determinative mechanism which persists as the established constitution of the germ.

Have the genes in question come into existence through the intermediation of the body, and if so was the influence general or specific? Are they attributable directly or indirectly to the external physical environment? Have they arisen as the result of internal constitutional changes which bear no direct relation to the body? Or are they the outcome of now one, now another, or of combinations of these agencies? These are the fundamental questions which confront us and which are likely long to confront us.

When one leaves the cemented highways of text-books and popular treatises on heredity, along which he glides from generalization to generalization, and seeks to penetrate the thicket of results from actual study of embryogenesis in individual organisms, he is likely to get a considerably modified conception of the separateness of the germ-cell from the soma. He finds that instead of a convincing demonstration of universal direct descent of the germ-cells from the original oosperm, actual visible proof of this has been brought forward in a very limited number of cases. Of these the highly specialized pedogenetic fly, Miastor, is one of the most outstanding examples.

Among plants and the lower animals, of course, it has long been recognized that it is futile to try to discriminate sharply between germplasm and somatoplasm because the process of propagation by budding, the facts of the regeneration of removed parts and similar phenomena abundantly attest the reproductive capacity of the somatic cells.

While inferentially, in the fairly limited number of cases in the vertebrates which have been investigated to determine the origin of the germ-cells, these cells are set apart from the somatic cells in early embryogeny, the evidence is by no means indisputable. The best that can be said is that in most of these cases the primordial germ-cells are recognizable early in the embryo, and that whether they come directly from the original oosperm or not, they arise at least from embryonic cells which have not differentiated into adult somatic tissues. But whether they arise from endoderm, mesoderm, mesenchyme or peritoneum is still in dispute.

Of vertebrates, the amphibians seem to have been worked upon most extensively. To realize how conflicting the testimony is one has only to read Professor George T. Hargitt's recent paper[2] on germ-cell origin in the newt, *Diemyctylus viridescens,* in which he reviews and discusses this field.

But whatever the origin of these primordial germ-cells may be, the evidence is by no means complete that they become the functional germ-cells. Various observers agree that in the amphibia there is extensive degeneration of the primordial germ-cells and that the origin of the functional germ-cells which succeed them is, to say the least, uncertain. Some would derive them from primordial germ-cells which had persisted in spite of the general disintegration of their kind, while others think the evidence shows derivation from adjacent tissues. Gatenby,[3] for instance, in his 1916 study finds that although the primordial germ-cells of Rana temporaria are entodermal in origin, they are of minor significance, for the greater number of definite germ-cells arise from the peritoneum, and he expresses the opinion that any peritoneal cell may become a germ-cell. Swingle[4] in his 1921 study finds that no germ-cells are detectable in larvae of the bullfrog (*Rana catesbeiana*) under 7 mm in length. At this stage they arise from entodermal tissue dorsal to the archenteron and keep multiplying until the larvae reach a size of 40 mm. The primordial germ-cells then degenerate for the most part, and a second generation is produced

[2] *Jour. Morph.,* September, 1924.

[3] *Quart. Jour. Mic. Sci.,* 1916.

[4] *Jour. Exp. Zool.*

which becomes the functional germ-cells of the adult. This second generation, he thinks, comes from a few undegenerated survivors of the original lot.

In the light of all the earlier papers on the source of the functional germ-cells in the embryos of various amphibia and of the successive supplies of spermatozoa in the testicular cysts of urodeles, Hargitt[5] has made a study of the origin of the spermatogonia of adult newts. He finds that in Diemyctylus when the cysts are once emptied they are generally without residual cells and "that there is abundant evidence of the formation of spermatogonia from the epithelial cells of the collecting ducts such as McGregor found in Amphiuma." He even finds isolated nests of germ-cells occasionally in the mesenteries and peritoneum outside the testes and he regards them as probably modified peritoneal cells.

To meet this situation, one way of cutting the Gordian knot, if we still find it necessary or expedient to talk about germplasm, is to follow the lead of those who have come to regard the germplasm as essentially the chromatin material of the cells. Since, through mitosis, this has descended from the original oosperm, obviously all cells have it, though ordinarily only the cells specialized as reproductive cells pass it on in the form of a new generation.

The production of double monsters, of identical twins or of several individuals from one original oosperm, as in the armadillo, indeed, lends further color to the view that all the descendants of the fertilized ovum, germinal and somatic cells alike, retain the hereditary potencies that existed in the original zygote. It has been shown repeatedly through experimental embryology and experimental morphology that in all probability a particular cell takes on the characteristics of a specific tissue, not because it differs constitutionally from other cells, but because of the differential nature of the stimuli to which it has been earlier exposed, or to which it is subjected as a result of its special location in the developing organism. The various phenomena which make up the field of experimental morphology to-day—heteromorphosis, regeneration and regulation, experimentally produced changes of axial gradients, dedifferentiation and redifferentiation, metaplasia and compensatory substitutions—all alike bear witness to the fact that the same initial cellular materials may yield very different end-products under different conditions; that many, possibly all, cells retain the fundamental constitutional mechanisms of the original oosperm.

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[5] *Jour. Morph.*, September, 1924.

Since this is true, then the argument commonly advanced against the possibility of a change in such a structure as brain or muscle being specifically paralleled in any degree in the germ, because the germ is a cell which does not possess a brain or a muscle, is largely beside the point. For brain or muscle is, after all, made up of cells each possessing hereditary potencies similar to those of the germ-cells. Any influence therefore that can induce changes in the fundamental constitution of a brain cell or a muscle cell, particularly if it were applied through the circulation media of the body, might conceivably affect the corresponding elements of the germ-cell. If an injury to or a change in a given tissue, for example, induces serological effects which react on the tissue itself, it seems permissible, without doing violence to the possibilities of the case, to suppose that the germ-cells might be also specifically affected.

Two obvious possible sources of influence from the blood stream, apart from the customary nutritive flux, are the internal secretions and the antibodies of various types which may come to occupy the blood.

The whole field of endocrinology is so much in the foreground at present that it is superfluous to enter into lengthy citations. The pronounced effects which may result in even a distant part of the body from the under- or over-secretions of some endocrine gland are known to you all. Of these none is more interesting to the biologist perhaps than the striking modifications of secondary sexual characters that have been brought about in the various castration, transplantation or injection experiments which are being reported in our present literature.

As an example of how far-reaching such experimental alterations may become I may cite the experiments of my associate, Dr. Hisaw, who in the pocket-gopher (*Geomys bursarius*), through the injection of ovarian material into males, induced in them absorption of a bar-like part of the pubic symphysis. This absorption occurs regularly in young females as the breeding season approaches, but the pubis of the male normally persists throughout life.

Concerning the possibility of antibodies affecting the germ-cells I have already spoken several times before this society, and I shall not weary you by repeating the details of the experiments which have led me to believe that they may be a source of germinal influence. With your indulgence I shall merely recall a few of the outstanding facts.

Two wholly separate lines of defective-eyed rabbits were secured by immunizing fowls against rabbit-lens and then injecting the blood-serum of such fowls into pregnant does. A third unrelated strain was secured by directly injecting rabbit-lens into the pregnant mother. In all cases the defects first appeared in the young of such treated mothers, not in the mothers themselves. As reported in our papers on the subject we had many more negative than positive results. Once the defects were engendered they proved to be hereditary. In the oldest strain they have been transmitted through nine generations. The defects included not only opaque or otherwise abnormal lenses, but also occasionally, microphthalmia, detached retina and other noticeable malformations. Of over 100 of the eyes which have been carefully mounted and studied by an ophthalmologist, Dr. F. A. Davis, every one showed some degree of coloboma. It is not unreasonable to believe, however, though it can not be proved, that the defects may all be traceable to initial lens-disorder which induced aberrancies or suppressions of normal eye-development. In some instances Dr. Davis has found that certain individuals of our defective-eyed strains which we had recorded as normal, were really abnormal in that the lenses were large and spherical instead of somewhat flattened as they are when under normal tension in normal eyes. One of my research assistants, Mrs. Mabel G. Boyden (paper in press), has shown by means of careful tonometric methods, furthermore, that in some individuals of the defective-eyed strains, even when defects are not visible, there may be abnormal intra-ocular pressure.

We have also secured lens defects on two separate occasions in the young of guinea pigs by treating the pregnant mothers, one with pulped swine lens, the other with pulped rabbit-lens, but inasmuch as these young died before reproducing we can say nothing regarding the inheritability of the condition in guinea pigs.

Recently we have gone about our experimentation in a different manner. We found that when its lens is injured a normal rabbit will develop lens antibodies in its own blood-serum. In such operations the eye is treated with a local anesthetic, then pierced with a needle by means of which the lens is more or less broken up. The procedure is the same as that commonly followed by ophthalmologists in cases of children with congenital cataract.

The serum of all rabbits used in such experiments was first tested for rabbit-lens precipitins and without exception found negative in all dilutions commonly employed in making precipitin tests. In from 7 to 10 days after the needling operation the serum of each rabbit was again tested for lens precipitins. Out of 14 rabbits thus treated 4 gave negative, 2 doubtful and 8 positive results. The lowest positive titer was a dilution of 1 to 40, the highest, 1 to 1,280. Some of those which were negative after the first operation, however, gave positive precipitin reactions after a second operation.

Inasmuch as an individual can evidently develop antibodies against its own injured tissues the question arose as to whether or not transmitted defects, similar to those of our earlier experiments, could be secured by this method. With this in mind we have at present a number of individuals under experimentation. Our experiments are of too short duration to be reported in any detail yet beyond relating that two different females (female No. 10 and female No. 26) with needled lenses have produced young with opaque lenses. The parents were normal animals bred in our own laboratory from stock we are testing out by inbreeding for genetical purposes. The first litter (nine in number) of female No. 10 by male No. N1A5 were all normal-eyed, notwithstanding the fact that the parents had had their eyes needled before the mating. The eyes of each parent were reneedled before the second mating. There were eight young in the second litter, every one of which had white chalky-looking lenses or lenses with white patches. Unfortunately these young have all died. Their eyes have been mounted, however, by Dr. Davis, and unlike the affected eyes of the earlier defective-eyed strains not one of them shows coloboma. The defect is more exclusively a lens defect.

Female 26, after the first mating to male N10A2, likewise gave birth to a normal-eyed litter, and similarly her eyes were reneedled. The eyes of the male were not needled for either mating. A second litter of four was born to 26, but she did not care for them well and only two lived to open their eyes. The lenses of these two, however, were opaque. So far five other females similarly operated upon have borne young, but none of these young have shown eye-defects.

Whether or not this latter method of experimentation will yield heritable defects can not be determined, of course, until the experiments are carried further. In any event, they give us just that much additional evidence that unborn young are open to serological influences from the mother. Furthermore, the operation is more nearly the kind of thing that might happen in nature than is the introduction of an alien serum into a pregnant mother.

Our published results, or more particularly our tentative conclusions, have not gone unchallenged. Most of the criticism has been justifiable, intelligent and helpful. Unfortunately—for the experimenters at least—the work is of such a nature that it has, or seems to have, bearings on a favorite theme for debate, upon which every argumentative person, from Pullman porter to professor of Greek, apparently claims sovereign right to an authoritative opinion; namely, the inheritance of somatic acquirements, or, as it is usually ambiguously stated, the inheritance of acquired characters. Needless to say we can not sponsor some of the uses—or better, misuses—to which our results have been turned in this connection. I raise this point simply because at different times I have seen such statements of what allegedly we "claim," or what we are "trying to prove," that I feel justified in offering a word of protest.

As a matter of fact, inspection of our published papers will show that we "claim" nothing beyond the right to make a few tentative suggestions as to the possible significance of our results. In the main we have been, and shall remain, content to record the facts as we find them. As to what we are "trying to prove" it should be needless for an experimental scientist to have to explain that he is not trying "to prove" anything, but merely attempting to find the facts in the case. To do this most expeditiously, to be sure, he may proceed by formulating various working hypotheses.

Let us now turn briefly to some of the more constructive criticism.

Professor Stockard[6] reiterates the fact that I myself have pointed out in practically all my papers on the subject that because of the unusual susceptibility of a developing eye to almost any kind of adverse influence, one might well question the specificity of the origin of a fetal eye-anomaly. From the very beginning of our work I have been inclined strongly to interpret the condition as the result of some general rather than of a specific influence, but the reason I can not accept this explanation as valid is the lack of evidence. The evidence from our controls all points toward specificity. We have had well over a thousand young born from mothers which had been subjected to just as violent or even more violent serological treatments in these and other experiments and never in a single instance have such young had defective eyes. Eye abnormalities have occurred only when a foreign serum which contained lens antibodies was used, or when the mother's own serum carried antibodies developed against lens injected directly or against her own lenses following their injury. Speaking of our experiments, Professor Stockard regards "the doses used as slightly less than a fatal amount," and he seems to think that in some way the foreign serum penetrates the placenta and is toxic to the young. He asserts, for instance, that we have "interpreted the result as a specific response of the germplasm to the lens antigen," when as a matter of fact we interpreted our results as probably a specific response to a lens *antibody*—a very different process. In our experience, although we have repeatedly tried, we have never, with one doubtful exception, succeeded in getting an antigen to penetrate the placenta. We have established, however, that at least certain classes of antibodies do penetrate the placenta. We have a paper in press at present on this aspect of the work.

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[6] Am. Nat., LVIII, 23–35, 1924.

I would not, however, take the position that antigen may not, as the result of accident, occasionally enter the fetal blood-stream. We have had a student, H. W. Mossman (paper in press), working for three or four years, by double-injection and histological methods, on this very point of the finer structural relations between the fetal and the maternal blood-vessels in the rabbit placenta. As a result of his studies and those of earlier investigators it is clear that after the twelfth day of pregnancy there are areas in which the maternal and the fetal blood streams are separated by only a single thin layer of fetal epithelium, and from the 22nd day on these areas are extensive. It seems not improbable, therefore, that as a result of the violent struggling a rabbit sometimes does when improperly picked up, this thin epithelium might be ruptured in places in the pregnant doe and thus permit more or less intermingling of the two blood streams. However, while apparently this might easily occur, I have no evidence that it does.

Finally, as regards specificity of effects, it might be pointed out that because a certain type of defect may result from various general adverse agents or conditions, it does not follow that it may not also result from a specific influence.

Huxley and Carr-Saunders,[7] in a paper entitled "Absence of prenatal effects of lens antibodies in rabbits," report the results of experiments in which they have rather closely though not fully paralleled our technique. Their results were negative with the possible exception of one very doubtful case. Twenty-nine does were treated with fowl-serum which had been immunized against rabbit-lens, ox-lens or rabbit- and ox-lens. Twenty-one of these bore fifty-seven young, all normal, with the possible exception already mentioned. Passing over other differences in technique I will only point out that they appear to have used the lenses of adult or nearly mature animals as antigen, while in the main we used the lenses of very young rabbits. Older lenses are tough and are even partially pulped only with difficulty, whereas the lenses of young rabbits go into almost complete solution or suspension. There may have been quantitative or even qualitative differences involved, therefore, in the original antigen.

Incidentally, it may be pointed out that some of their dosages were far in excess of the ones we used, so that if a general poisonous effect of a foreign serum were responsible for the production of eye-defects in unborn young, as Stockard believes, then some of these might well have been expected to have shown such defects.

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[7] *Brit. Jour. Exp. Biol.*, I, 2; Jan., 1924.

Again, Huxley and Carr-Saunders injected three does directly with rabbit-lens and six with ox-lens. Six of these died. The other three bore 17 young, all with normal eyes.

Negative results always have a certain value, of course, in experimental work where the conditions of the experiments are fairly parallel. If negative results alone, however, are the significant thing, then in the aggregate we can show many more than do Huxley and Carr-Saunders, for we have had far more failures than successes. All of which shows that the condition, in its visible form at least, is not an easy one to induce. Many complex factors are involved in such experiments and it is extremely difficult or even impossible, to secure similar conditions in all respects. The question seems to resolve itself into—not how much chaff you get—but is there any wheat? We have secured positive results with sufficient frequency to make it reasonably sure that the condition is not mere accidental coincidence. We have checked adequately against the possibility that it might be the uncovering of a Mendelian recessive which had in some mysterious way become incorporated independently in our several distinct strains of experimental rabbits. For even supposing that the improbable happened—that in our first experiments we just chanced to hit upon two individuals which were carrying recessive eye-defects, ready to be revealed in their offspring independently of our treatments, it is still more improbable that a similar defect would happen by chance in our unrelated 16A1 line; and the theory of chance, it seems to me, becomes wholly untenable when we attempt to carry it over to still a third unrelated strain, the 84 line, to say nothing of our later production of congenital cataract by lens-injury in parents, in still two other wholly unrelated varieties of rabbits. So that whatever may be the final explanation of just what has taken place in the germplasm, we feel assured that the results are in some way the direct outcome of our experimental treatment.

It has been objected that we used albino rabbits and that their eyes are already defective to the extent at least of being devoid of pigment. In answer to this I may say that neither of the last two does were albinos, in the young of which opaque lenses have been induced, nor was one of the guinea pigs. The defect once engendered, furthermore, can be crossed into pigmented as readily as into albino strains.

The fact that defective-eyed strains of rats, mice and guinea pigs have appeared from time to time in various laboratory stocks has also been argued as an objection. Until it can be shown how such so-called "spontaneous" anomalies originate, however, I fail to see just how they constitute a valid objection. They certainly do not arise uncaused.

In a recent paper[8] Little and Bagg raised the objection that an outcross of an abnormal male with an unrelated normal female, followed by extraction of the defect through the breeding of such progeny to the same or to another abnormal male, does not prove inheritance, since, to quote them, "the direct use of an abnormal male involves the possibility that transmission of an agent producing an abnormality might be brought about by the seminal fluid or other material transferred with this sperm at copulation," and they imply that our pedigrees are inconclusive in this respect. They go on to say: "The most satisfactory type of test is an outcross of a normal male with unrelated normal females followed by brother-to-sister inbreeding of the normal progeny from this cross." However, if they will but examine our Fig. 4, on page 463, *Journal of Experimental Zoology*, for January, 1924, they will find that we have done even better than this. A defective-eyed male (3A1) was mated to a normal unrelated female (11). Two of the normal-eyed daughters (53A2 and 53A1) were mated respectively to normal unrelated males (25 and 55A1), and a normal-eyed male offspring (90A2) of one of these matings (25 × 53A2) was finally bred to a normal-eyed female offspring (91A4) of the other mating (53A1 × 55A1) with the result that in the ensuing litter of six two defective-eyed individuals—one male and one female—reappeared. In other words, the defect introduced through male 3A1 was made to reappear in his great-grandchildren after it had passed in a recessive condition through two normal-eyed females in one generation and a normal-eyed male and normal-eyed female in the next generation. Moreover, pigmentation was introduced into the stock through male 25, and these great-grandchildren of 3A1 not only show marked eye-defects but show them in pigmented eyes. In our published papers, inasmuch as we were concerned primarily with other than genetical data, we have shown only a few of our pedigree charts. Upon looking over our records, however, I find still other cases which fulfill the requirements specified by Little and Bagg.

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[8] *Jour. Exp. Biol.*, 41, 1; Nov., 1924.

When one takes into account all the facts, therefore, there can be no reasonable doubt, I think, of the true hereditary nature of the anomaly, nor that it has been engendered in some way by means of our serum-treatment. As already pointed out, in spite of one's general inclination from knowledge of the field of experimental embryology to attribute the initial defect to a general poisonous or inhibitive agent, all our own results checked by over a thousand controls point to it as specific rather than general. We have never obtained the defects in question except in association with a serum carrying specific antibody. But even should the defect have originated from a general rather than a specific cause, the germinal basis must have become specific since the anomaly reappears generation after generation without any recognizable accompanying malformations of other parts of the body. In my opinion, however, it is not without the range of possibility that an eye-defect, however engendered in the first place, might lead to the development of antibodies which could inaugurate specific changes in the germinal correlatives of the somatic parts affected.

Are our results an example of the inheritance of a somatic acquirement? We have never maintained that they are. It is not yet clear whether the eye of the fetus is first changed and the condition is then conveyed from it to the germ-cells of this individual, or whether the eye and the germ of the fetus are influenced separately by the antibodies. Obviously only the first occurrence could be interpreted as the inheritance of a somatic change; the second would be an instance of parallel induction. Strictly interpreted, even in the defective-eyed young of mothers with needled lenses, supposing the condition proves inheritable, the same two alternatives confront us. Until adequate outbreeding experiments with needle-eyed males have been made the question must remain unanswered. Such experiments are in progress.

Admitting, as I think we shall have to, that the distinction between soma and germ has often been overemphasized, we shall have to recognize that in germ-cells we are dealing with protoplasmic complexes not essentially different from those of the somatic cells. And it follows that we can not look on germ-cells any more than on somatic cells as insusceptible to the ebb and flow of the chemical or other physiological influences within the body. Should the germplasm prove to be thus open to modification, this does not, of course, carry with it the implication that all changes in it are initiated in this way, or that the main current of inheritance is due to somatic influences. Even granting the possibility of somatic interference there is no reason for supposing that genes once established would not persist indefinitely in the germ unless the somatic changes in question have some direct bearing upon them.

Apart from specific influences, however, recognition of the close physiological dependence of the germ on the rest of the body makes it easier for us to conceive of how many influences might be brought to bear on the germ-cells which might lead to constitutional germinal changes not specifically related to the impinging cause. Such a conception would seem to open the way more clearly to a rational explanation of the origin of variations. Even Weismann, in his theory of germinal selection, since the food of his hypothetical components must come from without, had virtually to admit that the inception of germinal variation as he conceived it was environmental in origin.

The fact is that biologists have never yet hit upon an explanation of the marvelous adaptedness of organisms to their environment which does not put a tremendous strain upon our credulity. And when we view it with unprejudiced eye it seems to me that the theory which would attribute adaptation to the mere accumulation of such chance variations as happen to be favorable is the most incredible of all. Time and again in the past, according to paleontologists, whenever new possibilities for existence occurred, forms of life admirably adapted to those conditions have come to occupy the new habitat. In some way the environment has molded these new inhabitants to its bounds, and it takes more faith than I personally possess to believe that it has all been done by the negative method of killing off, generation after generation, the non-conformists—those in which the happy accidents, or rather the innumerable interrelated series of accidents, has not occurred. It seems more within the bounds of credence to suppose that biologists have failed as yet to discover some more direct principle of adaptation whereby living organisms are fitted to their environment. And since in the higher animals, at least, we find abundant evidence of compensatory and other adaptive somatic responses initiated through the agency of the serological or humoral mechanisms of the organism, it would seem well worth while to seek in the same direction for light regarding the possible existence of some form of adaptive variation. For the germ-cells no less than their somatic brethren are open to the surge and sweep of the circulating fluids of the body.

SEX DETERMINATION AND SEX DIFFERENTIATION[1]

SEX DETERMINATION AND SEX DIFFERENTIATION IN THE HIGHER PLANTS

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WHEN Mendelian heredity became definitely known, it was quite natural, of course, that the question as to the Mendelian nature of sexuality should present itself, and since it was well known that in the higher animals and some diecious plants the ratio between the two sexes is about equal, the hypothesis of homozygous and heterozygous constitutions was soon formulated. The basis for such a hypothesis seemed quite secure because of the known fact of unequal pairs of allosomes in the higher animals. Thus the hypothesis of sex-determining factors or sex-determining chromosomes has become to many a settled conviction. But to a botanist who has to deal with sexuality as it is actually manifested in the vast majority of gametophytes and heterosporous sporophytes, the phenomena of male and female sex, both as to determination and differentiation, appear to lie mostly beyond the processes of chromosome and factor shiftings and take on a physiological aspect for the simple reason that in the usual type of gametophyte and sporophyte there is no sex determination either at the time of fertilization or of reduction. It is evident to any one acquainted with the animals, also, that the problem does not fit into any such simple strait-jacket as a Mendelian formula would require. The matter presents itself in sharp contrast to ordinary hereditary phenomena, which resolve themselves into a multitude of minute genes or factors of the most diverse kind, while sexual phenomena are universally manifested in but two general conditions as male or female.

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[1] Symposium on Sex Determination and Sex Differentiation presented before the meeting of the American Society of Zoologists at Washington, D. C., December 31, 1924.

Now every specific sex character must be inherited in the same way as any other character not influenced by the sexual states. The genes of such characters are subject to aggregation and segregation in fertilization and reduction, to displacements and crossing over, the same as any other factors which are not especially influenced by sexual states. The problem of sexuality does not lie there. Many experiments have shown that the sexuality, whether expressed as a male, female or neutral condition, of a cell, tissue, organ or individual belongs in the domain of physiological states of the protoplasm and that it is apparently conditioned and determined by physiological gradients. If this is true then sex determination and sex reversal may be brought about not only in the manner usual for the given species or given hereditary complex acting in a given environment but also in various other ways and at other times of the life cycle, whenever a proper change in the functional state is produced through environmental or ecological factors.

In sexuality, we have a condition in the structural protoplasm or in its inclusions corresponding in some way to positive and negative chemical or electrical states so very common in the inorganic world, but the real nature of these conditions or states is yet to be investigated. But in the meantime, the phenomena of sexuality can be properly classified and the ecological basis of determination and control discovered to a considerable extent before we have solved the more hidden processes and conditions on which a specific female, male or neutral expression depend. Genetics is then not the primary basis for an investigation of sexuality but is a merely incidental phase which must be taken into consideration in studies on the higher animals and a very few plants. It is useless to speak of homozygous and heterozygous conditions as determining sexuality or its reversal when in the vast majority of plants neither sex determination nor reversal occur at the time when the chromosomes with their Mendelizing factors are associated in fertilization or segregated in reduction.

The evidence from plant taxonomy for the view that sex determination and sex reversal are not at all dependent on or even to any extent associated with differential hereditary constitutions is overwhelming, but lack of time forbids the presentation of such evidence here.

The terminology of sex is not very definite and much confusion no doubt exists because of a different use of terms. We distinguish sexual states as distinct from the characters expressed by the hereditary factors when such physiological states are present. These states are of various degrees of intensity and persistency. Sexual states are primary or secondary. Primary states apparently arise only in gametes which are entire cells or nuclei or in the synaptic chromosomes during reduction and the main characteristics of such a primary state are the properties of attraction and fusion. For convenience, dimorphic characters of the gametes are called primary sexual characters without reference as to the actual cause of their origin which may perhaps be due to secondary sexual states the same as in the case of secondary sexual characters. Primary sexual states may arise in typical, isogamous or heterogamous gametes or in only slightly modified cells as in the conjugatae and typical fungi. The attractive or sexual states which arise in the synaptic pairs of chromosomes may occur at three different points of the life cycle; first, at the germination of the zygote, usually followed by a haploid sexual generation; second, just before the formation of gametes, usually followed by a diploid sexual generation; or third, in an alternation cycle, at the end of the normally diploid sporophyte when non-sexual spores are produced which give rise to a haploid, gametophytic generation. Secondary sexual states arise in cells outside of those destined to become gametes but do not attain to the property of attraction and fusion. They may, however, have a profound dimorphic influence on the expression of certain hereditary characters of the cell, thus giving rise to secondary sexual characters. Some cells in the primary sexual state may pass by parthenogenesis into a secondary or neutral state again.

In studying sexual phenomena, a clear understanding of the sequence of events in the life cycle is of fundamental importance in order to understand the succession of functional states, the changes of sexual conditions and their relation to the shiftings of chromosomes and other cell structures. In making such a study of the plants with a typical antithetic alternation of generations, it becomes evident that there is normally but one stage of sex determination in the life cycle, which stage may, however, consist of a series of male and female determinations or their reversal. If the sex is determined in the gametophyte, which is usually haploid, there is no maleness or femaleness in the sporophyte. If the sex is determined in the sporophyte, which is usually diploid, it remains unchanged through the reduction phase, both sides of the reduction being of the same sex and the following gametophytes are normally always unisexual. Roughly speaking, one may say that there are about twelve main points in the life cycle where sex determination may take place. Of these twelve successive points of sex determination, there are two in which the time of determination coincides with chromosome shiftings. In plants with unisexual gametophytes and homosporous sporophytes, the time of sex determination coincides with reduction; in plants with unisexual gametophytes and diecious sporophytes, the time of sex determination apparently coincides with fertilization, although it is possible that in this case the determination takes place in the egg before fertilization or some time after fertilization is completed. That the determination of sex in the case of unisexual haploid gametophytes is a mere coincidence in most cases can be shown by such examples as the ostrich fern and the common horsetail where the sex as determined may be easily reversed in the mature individual, the female to the male and the male to a certain extent to the female.

To determine the nature of the sexual condition in the higher plants, I used various diecious species, and since the sporophytes of the higher plants are extremely indeterminate in development, they make unusually favorable objects for experiment. The same is true of the indeterminate gametophytes of the Bryophytes and some Pteridophytes. But the higher animals, aside from the possible influence of the apparently sex-producing allosomes, are very unfortunate objects for such experiments because of the decided determinate development of the individual and most of its organs. Yet in spite of these conditions practically complete, functional sex reversals are being reported especially in the case of birds. In my experimental work on sex control and reversal, some species have proved very plastic and amenable to control, while others showed only a slight response to the ecological factors employed.

In *Arisaema triphyllum* (L.) Torr., which is a diecious plant with a variable percentage of intermediates, sex was almost absolutely controlled in the individual through a control of the nutritive environment. Abundance of moisture and manure with normal leaf surface throws the staminate and intermediate plants into the female condition and continues the carpellate individuals in the same condition, while a rather dry environment with a poor or ordinary soil and a reduced leaf surface throws the carpellate and intermediate plants into the male condition and continues the staminate plants in the same condition. These reversals can be produced back and forth repeatedly. *Arisaema dracontium* (L.) Schott. has a hereditary constitution that under the normal environment gives rise to staminate individuals and monecious individuals. I have never found pure carpellate individuals, although a few cases approached such a condition. Under the usual environment of the species, there is always a great excess of staminate plants. The species is preeminently a masculine species. Experiments of the same nature as those carried on with *Arisaema triphyllum* showed that the staminate individuals change to monecious individuals and monecious individuals change to staminate individuals.

Extensive experiments on the common hemp (*Cannabis sativa* L.) have shown that sex reversal results by changing the length of the daily illumination period. Hemp planted at the normal time in early spring develops as pure carpellate and pure staminate individuals with about equal numbers of the two sexes. With the variety used in the experiments no intermediate individuals whatever appeared under the normal environment. But when planted in rich soil with changing light period from August 15 to December 25 and from this date to May 1, the plants showed increasing sex reversal in both directions, depending on the relative length of daylight. The amount of reversal is approximately inversely proportional to the length of daylight. This percentage can be predicted just as definitely as any Mendelian ratio, when the proper environmental conditions are present. It is to be especially noted that in plants like hemp the same ecological factor or factor complex, contrary to what occurs with the nutritive control of Arisaema, produces reciprocal results, the female condition is reversed to the male condition in the carpellate plants and the male condition is reversed to the female condition in the staminate plants. There is also apparently a gradient of fixity or degree of intensity of the sexual state in the various individuals of a progeny which causes them to succumb to the environmental influence in the order of their several intensities, the weakest presumably responding first to the external stimulus. Thus in a patch of carpellate plants, some reverse promptly and some only after several months.

The percentage of intermediates or individuals that showed sex reversal to a greater or less extent among the carpellate plants for the successive planting dates was as follows:

Date	Percentage showing reversal	Total number of carpellate plants
August 15	0 per cent.	—
September 20	38 " "	72
October 19	53 "	63
November 1	76 "	120
December 1	80 "	73
December 15	86 "	29
February 1	65 " "	70
March 15	34 "	59
May 2	0 "	30
May 20	0 "	24

The highest percentage of reversal for carpellate plants was obtained in two plats planted on December 19, 1919, in a north side room of the greenhouse where. in addition to the short period day, the light was less intense and the temperature somewhat cooler. One plat had 18 carpellate plants, 16 of which showed reversal and the other had 36 carpellate plants, 32 of which showed reversal. The sex reversal for these carpellate plants was, therefore, 88 per cent.

For the staminate plants the percentage of reversal was as follows:

Date	Percentage showing reversal	Total number of staminate plants
August 15	0 per cent.	......
August 19	15 " "	120
September 1	35 " "	20
September 20	57 " "	21
October 19	67 " "	40
November 1	90 " "	116
November 21	73 " "	73
December 1	93 " "	66
December 15	93 " "	[illegible]
December 20	90 " "	[illegible]
January 20	68 " "	[illegible]
February 1	65 " "	72

Date	Percentage showing reversal	Total number of staminate plants
February 20	56 Per cent	37
March 15	60	50
March 20	33	30
April 20	0	28
May 1	2	93
May 2	5	18
May 20	0	32

These records are from plantings covering several years, and the available daylight for any given period may be considerably less in one year than in another, which may account for the slight discrepancies in the table of staminate plants. The 93 per cent. reversal is the highest percentage obtained for staminate plants.

In the process of sex reversal from female to male or *vice versa,* the physiological condition must necessarily pass through a neutral point. This results in the abundant production of bisporangiate flowers, monstrosities and intimate sex mosaics in such hemp plants. These abnormalities appear also in the Arisaema intermediates and various other species used in the experiments.

Relative length of illumination period has similar effects on Japanese hop (*Humulus japonicus* Sieb. and Zucc.). As in the hemp, the same environmental complex causes reciprocal reversal and also the production of bisporangiate flowers, abnormal organs and intimate sex mosaics.

Thalictrum dasycarpum is a diecious plant which in its normal habitat shows sex intergrades quite frequently. These intermediates are of every conceivable degree, ranging up to individuals producing about an equal number of staminate and carpellate flowers or sporophylls. The question presented itself as to whether this series of sex forms represents so many types of hereditary constitutions or chromosome or factor arrangements which are responsible for the result. A simple experiment answered the question. Pure carpellate and pure staminate individuals were transplanted from their rich flood in habitat to rather dry clay soil. In two years both pure carpellate plant and the pure staminate plant developed into sex-intergrades. The carpellate plant developed decided maleness and had many more staminate flowers than carpellate ones. The notion of the fixity of the sexual state, dependent on a specific heredity complex, was thus shown to be entirely erroneous in this case also. The cause of these different grades of sexual expression is not found in specific hereditary constitutions. The various types of intergrades as well as the pure individuals apparently have the same hereditary constitution which is of such a nature as to permit sexual states to be easily changed.

Numerous other examples might be given from my own observations and experiments as well as from those of other investigators, but time permits of but one further point in the discussion.

If the physiological view of sexuality is the correct one, it becomes evident that cells or cell lineages must be able to pass from one sexual state to another without change of hereditary constitutions and such is actually the case. In the dandelion (Leontodon), various varieties or species have evolved to a condition where normal synapsis does not take place except in rare instances. Sears and others have shown that the plant usually passes through its life cycle from generation to generation by means of parthenogenesis with the diploid number of chromosomes. Beginning in such a case with the vegetative cells of a sporophyte, such cells are in a neutral, vegetative state in respect to sex. When flowers appear, secondary sexual states arise and secondary female characters are developed in the gynecium on the one hand and secondary male states and secondary male characters in the andrecium on the other. In the ovules the megasporocytes divide without reduction, the female gametophyte develops with the usual secondary female characters but with the double number of chromosomes. An egg which is then also diploid is organized, but whether a primary sexual state appears giving it the power fusibility can not, of course, be determined in any spe case of parthenogenesis. It is well known that in mo the double number of chromosomes is no barrier to the development of attractive properties and subsequent fertilization. The diploid egg develops the new, neutral sporophyte which again produces flowers. Passing through the andrecium cell lineage, the stamens show secondary male states and characters. In the microsporangia the microsporocytes may divide without reduction, with abnormal or incomplete segregation of chromosomes, or occasionally with a normal reduction, thus giving rise to normal microspores and male gametophytes with normal haploid spermatozoids. In this cell lineage, therefore, without any change in chromosomes or hereditary constitution, the sexual state passes from a neutral condition to a female condition, to a neutral condition, to a male condition.

Blakeslee and Belling have recently reported the interesting case of haploid sporophytes in the jimson-weed (*Datura stramonium* L.). They consider the evidence good that such haploid sporophytes arise through parthenogenesis. The haploid cell lineage will, therefore, show the same changes in sexual states as that of the diploid Leontodon lineage. Proceeding through the same tissues we have: neutral, diploid sporophyte, secondary female state and female characters in the gynecium, sporocyte, reduction (without change of sex), female gametophyte and primary female characters in the egg (and perhaps also primary female state for a period). Through parthenogenesis the neutral, vegetative, haploid sporophyte is produced which leads on to secondary sexual states in the flower, female characters in the gynecium, male characters in the andrecium. In rare instances normal, haploid, non-reduction divisions take place in the sporocytes leading on to normal male and female gametophytes, respectively, with normal sperms and normal eggs, which in consequence of the primary sexual states arising in may conjugate and produce a diploid generation in. Thus it is seen, as is perfectly plain from the onomic evidence, that the presence of a diploid or loid number of chromosomes has nothing to do with origin of primary and secondary sexual states and racters.

In my photoperiodic experiments with hemp such a case of definite sexual changes appeared in a rejuvenated carpellate plant. A certain short-light individual was pure female in expression and produced numerous normal carpellate flowers and seeds. After it became quite decrepit, it was rejuvenated from side buds by means of continuous light and passed into a new vegetative phase of growth. In passing through this reversed physiological gradient, after growing for about a foot, it began to bloom again and produced a considerable number of flowers and, as some would say, *mirabile dictu,* these flowers were all absolutely of the pure staminate type. The pure female had for the time being changed to a pure male, when judged by its reproductive behavior. It soon stopped blooming, however, because of the continuous light effect and the terminal end of the inflorescence continued a normal vegetative growth. After several months the light was turned off and it promptly passed into the blooming condition again. At this third blooming period, every flower was pure carpellate! After some seed had set it was cut back to some extent, rejuvenated again, and after a period of vegetative growth was beginning its fourth period of anthesis, but died, because of previous, improper pruning, before the flowers were fully expanded. Some of the flowers had well-developed stigmas, but none showed recognizable staminate structures, as might have been anticipated from the date of this blooming, December 15. If the plant had lived, some maleness might still have developed. Dandelion and jimson-weed are bisporangiate plants; here we have a strongly dimorphic, diecious species—sexually dimorphic in its vegetative organs and in its reproductive organs, and strongly

dimorphic in its physiological processes—a carpe
plant of which developed successively a vegetative tis
that at first produced secondary and then primary fem
states and characters, next secondary and primary m
states and characters, and then secondary and prim
female states and characters again. The successio
functional states leading to the several zones of sexu
expression is exactly similar to what takes place in numerous monecious species in which the flowers appear in a definite zonal succession, but with this difference in the vegetative parts. In normal monecious plants the vegetative phase is neutral; in this carpellate plant the vegetative phase was in the first period of growth typically female.

In organisms which have allosomes, the most that can reasonably be claimed for the allosomes is that they may be sex-producing. The given heredity complex may produce a higher or lower metabolic level and thus control sex. They can plainly not be sex-determining. But if they are merely sex-producing rather than sex-determining, then the sex of an organism or any of its parts containing such allosomes is subject to reversal through a control of its physiological states by external factors the same as in organisms without allosomes. Whether such reversal is more difficult to produce in allosome-containing cells is a question in view of the well-known fact that with a change of hormones in the circulatory system of some of the higher animals the sexual expression changes readily, and apparently to as great an extent as the determinate nature of the animal will permit. In view of such facts as normal hens changing completely or functioning as roosters, etc., it is evident that allosome-containing organisms are suitable subjects for physiological experimentation with a view to producing sex reversals in either direction the same as organisms without such allosomes, although it may perhaps be more difficult to produce the change. But the very fact that allosome-containing organisms change their sexual states and sexual expressions as readily as they do throws considerable doubt on the hypothesis that they are even primarily sex-producing. It may be a mere coincidence. They may be merely sex-indicating and after a case of sex reversal they can not even be that. The sex of the egg may be determined even before fertilization takes place. Many botanists formerly thought that a diploid number of chromosomes was the cause of sporophytic expression and a haploid number the cause of gametophytic expression, but we now know from a study of apogamy and apospory and from experiments on mosses, as well as from such haploid and diploid parthenogenetic developments as Datura and Leontodon, that the doubling and reducing of chromosomes in the higher plants not only has no direct relation to sex determination, but also that these processes have no causal relation in the production of the profoundly dimorphic gametophyte and sporophyte.

The conclusion is then that sex in organisms is primarily dependent on physiological states, that these states are subject to change and reversal through ecological factors and that, therefore, sexuality is a subject for experimental investigation and one of the problems that physiological ecology alone can properly solve.

SEX IN RELATION TO CHROMOSOMES AND GENES

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NG the three years since the report at the Toronto
(Bridges '22) considerable new information has
ed with regard to the series of different sex-
has arisen in the breeding work with Drosoph-
I). Each of these different sex-types is the
particular combination of chromosomes. They
ipally among the offspring of females that are
hat is, that have three X-chromosomes and

three of each kind of autosome. The possession of an extra X and at the same time of an extra set of autosomes does not change this individual in its sexual characters from the normal type of female. However, in gametogenesis the 3N group is an unstable one. Each egg receives a full set of chromosomes and a full set goes to the polar body. The members of the extra set are distributed between the egg and the polar body in all possible combinations. Thus, a common type of egg of a 3N female has an extra set of autosomes. If we represent a set of autosomes by A, this egg can be formulated as X + A + A or X,2A. When such an egg is fertilized by an ordinary sperm, that can be formulated X + A, the zygote is 2X,3A. This type of zygote develops into an intersex, that is, into an individual that is neither male nor female but an intermediate, or rather a mixture of male and female parts, very similar in type to the intersexes that Goldschmidt has worked with so extensively in *Lymantria dispar* (Goldschmidt '20).

TABLE I

RELATION OF SEX TO CHROMOSOMES IN DROSOPHILA MELANOGASTER

Sex Type		X (100)	A (80)	Sex Index	Interval	X = − 6 A = + 2
Superfemale		3	2	1.88	50%	− 14
Female	4N	4	4	1.25	......	− 20
	3N	3	3	1.25	......	− 12
	2N	2	2	1.25	.....	− 8
	N[1]	1	1	1.25	50%	− 4
Intersex	♀ type	2	3	.83	......	− 6
	♂ type	2	3–IV	.83	33%	− 6
Male		1	2	.63	50%	
Supermale		1	3	.42	......	

The interpretation of these intersexes in term
carried in the chromosomes was made possib
result of the very extensive studies of the n
which ordinary characters are determined, and
from the study of the contrasted characte

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[1] The haploid type has not been discovered.

brought about on the one hand by the loss, and on the other hand by the gain, of one of the small round chromosomes, the fourth chromosome. From such studies the view had been reached that each character of an individual is the index of the point of balance in effectiveness of a large but unknown number of genes, some of which have a tendency to change development in one direction and others in the opposite (Bridges '22). This conception of "genic balance" was applied to the sex characters of the intersexes as follows: In chromosome constitution the intersexes differ from females only in that they have an extra set of autosomes. This proves that the autosomes are concerned with the determination of sex. Moreover, they are male-determining in their action, since the addition of a set of autosomes causes the female to assume male characteristics. That is to say, in the autosomes there are genes that tend to produce the characters that we call male, and these are more effective, either through greater numbers or through greater potency, than the total of autosomal genes tending to produce the alternative characters that we call female. On the other hand, the X has a net female tendency, as shown by the fact that the addition of an X to a male group changes the individual into a female. The net male tendency of a set of autosomes is less than the net female tendency of an X. This is seen in the fact that in the individual in which there are two of each, namely 2X,2A, the female genes outweigh the male and the result is a female. If we represent the net effectiveness of the female tendency genes in the X by 100, then we should represent the net male effectiveness of a set of autosomes by some lower number; let us say 80. In a 2X,2A individual the ratio of female effectiveness to male effectiveness is 200:160, or 1.25 to 1; and on this formulation the sex index of 1.25 corresponds to the normal female. In the X,2A individual the ratio of female to male effectiveness is 100:160; or the sex index of a normal male is 0.63. In the 2X,3A intersex the ratio is 200:240, and the sex index is 0.83, which is intermediate between the indices for female and male. In the 3N female the ratio is 300: 240, and the sex index is 1.25, exactly the same as in the normal female. This identity of sex indices for the 3N and 2N forms corresponds to the observation that there seems to be no strictly sexual differences between them. The larger size, coarser texture of eye, etc., of the 3N can be directly attributed to the changed volume of the nucleus, and are not sexual in nature.

Another type of egg of the 3N ♀ is X + X + A; and this, fertilized by a normal XA sperm, gives a 3X,2A individual with a sex index of 1.88, which is 50 per cent. higher than that of the normal female. This constitution corresponds in fact to the "superfemales" that occur in these cultures and elsewhere. The superfemales are much delayed in development, are rarely able to live and are probably completely sterile.

Conversely, an X + A + A egg, fertilized by the type of sperm that does not carry an X, gives an X,3A zygote with a sex-index of only 0.42. This type of individual was expected to be more male-like than an ordinary male; and such individuals were looked for among the offspring of 3N females. At first none were found; but presently it was discovered that very late in the cultures an occasional example of a distinct type of male occurred. These so-called "supermales" are likewise sterile. Recently, cytological proof has been secured that this type of sex has the constitution X,3A, which agrees with the genetical evidence previously secured.

It was observed that the intersexes showed considerable variation and seemed to form a bimodal class. And since the cytological investigation had showed that some intersexes had three and others only two of the small round fourth-chromosome, it was guessed that the more male-like mode corresponded to the full trio of fourth chromosomes, while the more female-like mode corresponded to the cytological type that lacked one fourth chromosome. An effort has been made to secure cytological evidence on this point. But this evidence is inconclusive; as is also that from an attempt to make a genetic test of the number of fourth chromosomes present through use of the fourth-chromosome mutant character eyeless. At present extra fourth chromosomes are being artificially inserted into the intersexes by continually crossing 3N mothers to males known to have an extra fourth chromosome. Contrariwise, in other lines of intersexes, fourth chromosomes are being diminished in number by continually mating 3N mothers to males known to lack one of the two fourth chromosomes. Similarly, the superfemaleness of the 3X,2A individuals might be reduced or be increased by matings with triplo-IV males or with haplo-IV males. For this experiment females are being used whose two X-chromosomes are permanently attached to each other (L. V. Morgan '22), and hence that give through non-disjunction a very high proportion of 3X-superfemales. Present indications, from the uncompleted experiments, are slightly contradictory, but tend to a conclusion which is the opposite of that earlier reported as probable on the basis of the slight evidence then available (Bridges '22). When the number of fourth chromosomes is three the intersexes are more female-like, and when the number is two they are more male-like. The fourth chromosome has a net female tendency, similar to that of the X and different from that of the other autosomes. By variation in the number of fourth chromosomes it is possible to have a fringe of minor sex-types about each of the major types of sex difference.

The list of sex-types has been enlarged by the discovery of tetraploids, or 4N individuals. These are females, quite identical with normal females in sex characteristics. The tetraploid arose in a stock of triploids; and was detected only by the strikingly different offspring given. A female supposed to be 3N was selected from the 3N stock and outcrossed to a normal male. All the offspring were triploid females (about 30) or triploid intersexes (about 20). There were no 2N offspring or supersexes. It was seen that this result might be produced if the mother were 4N instead of 3N. For in that case all the reduced eggs would be 2N; and these fertilized by X-sperm would give 3N females, and fertilized by Y-sperm would give 2X,3A intersexes.

Before the discovery of this 4N individual, an expectation that it would occur had arisen from several facts. Thus, in the three years following the discovery of triploidy there had been found no less than twenty-five instances of the new occurrence of triploidy. This very high frequency was paralleled by cytological observations that give the explanation of the origin of triploids. In three separate preparations of ordinary 2N females it was found that a portion of an ovary was constituted of markedly larger cells; and in two of the individuals some of the giant cells were in division, and the chromosomes could be counted as 4N. Evidently there had been in some oogonial cell a division of the chromosomes that had not been followed by division of the nucleus and cytoplasm. The resulting tissue was tetraploid, and any reduced gamete would be 2N. Such a 2N gamete, fertilized by a normal sperm, would account for each of the twenty-five recurrences of triploidy.

Furthermore, in examining sections of intersexes, two individuals were found in which similar cysts of even larger cells were present. In one of these cysts divisions were occurring; and the chromosomes were clearly 6N. A 6N cyst in a 3N female would give, upon reduction, 3N eggs, which, fertilized by X sperm, would give the expected 4N type of female.

Soon after this first case of 4N female a second similar case was found. Also L. V. Morgan found a third case, and was able to prove by genetic tests that four separate X-chromosomes had been present (in press).

The fact that 4N individuals are females, not modified as to sex, has important bearings on our ideas as to the way in which genes interact to produce their effect. The view adopted here is that in general the effectiveness is in proportion to number of genes, and the significant point is the *ratio* between sets of genes that tend to produce alternative effects. On this view we find a ready explanation for the fact that such diverse forms as 2N, 3N and 4N individuals are precisely alike in their sexual characteristics; for in all these forms the effectiveness of both contending sets of influences has been doubled, trebled or quadrupled; and the ratio remains constant.

But a system of formulation different from the ratio type has been adopted by Goldschmidt in dealing with the intersexes produced in the course of his brilliant work on racial crosses of *Lymantia dispar.* To the male tendency of a particular race he assigns a positive value that is proportional to the strength of the male-determining gene or genes. To the female tendency he assigns another value also proportionate to the strength of the female-determining gene or genes. He then assumes that when in an individual the male value is greater than the female value by a certain number of units the individual is a male, and that, conversely, when the female value is greater than the male by this same number of units the individual is a female. The locus of the male tendency gene (M) is in the "Z-chromosome" of which two are present in the male and one in the female. The female tendency is strictly maternally inherited; and hence the locus of the F genes is in the W-chromosome that descends from mother to daughter. The F gene is supposed to exert its influence on the cytoplasm of the developing egg; and hence, although the male has no W-chromosome, he is supposed to have a definite female tendency that was impressed upon the cytoplasm of the egg and that persists throughout development. For a "weak" race the value assigned to F is 80, and to M, 60. In the WZ individual the cytoplasmic $\boxed{F}$ of 80 exceeds the M value of 60 by "the epistatic minimum" of 20 units, and the individual is a female. Likewise, in the ZZ individual the $\boxed{F}$ is 80, but the net M value is twice 60 or 120, with an excess in the male direction of 40 units. For a strong race both F and M are higher, for example, 100 and 80, but the arithmetical relation between the values of F and M would still govern the sex of the individual. In a cross between a weak female and a strong male the ZW individual received an $\boxed{F}$ of 80 from the mother and an M of 80 from the father. The values are thus balanced midway between the excesses necessary for a female on the one hand or a male on the other, and the result is an intersex. This far the formulation is satisfactory; but when extensive series of crosses are compared, and an attempt is made to give values to the F and the M of each race that will hold throughout the entire range of experiments, this attempt is rather unsuccessful. It seems to me that a reformulation on the ratio instead of the algebraic basis would give a series of consistent indices without running counter to the very valuable physiological ideas that Goldschmidt has developed.

In the table of sex-types of Drosophila the haploid individual is entered with the index 1.25, the same as that of the 2N, 3N and 4N females. Unfortunately the haploid individual has *not* been discovered. But it is plain from the view just given that the expectation for a haploid *Drosophila melanogaster* is that it would be female in sex character. Accordingly, it is necessary to assume that the sex-determining mechanism here is essentially different from that in the bee and similar forms in which the haploid individual is a male. To me, sex-determination in the bee is the outstanding unsolved puzzle, although before the development of the idea of genic balance it seemed one of the clearest and simplest of cases. If it is true that the male is a haploid individual, then one would suppose that the diploid individual should likewise be a male, since the ratios among the sex-determining genes are not different in the two cases.

Schrader and Sturtevant have attempted a reconciliation of the cases of Drosophila and the bee by use of Goldschmidt's algebraic formulation. They assign a positive value, *e.g.*, + 2, to each A and a negative value *e.g.*, — 6, to each X. It is then assumed that the effective relation is the algebraic sum of the values of X and A, as given in the column to the right in Table I. On this view the haploid might be a male. But this system has a difficulty in that the intervals between successive indices do not correspond very well with the observed differences between the sex grades. Thus the smallest observed interval in fact, that between the 3N and 2N individuals, is represented by a difference of 4 units, while the very great interval between the male and the female is represented by only six units. At that time the 4N type was not known; and when it is added to the series, the fit is very poor on the algebraic system and very good on the ratio system. I repeat that I do not regard the case of the bee as interpretable on the same basis as Drosophila so long as the present account of the mechanism for the bee is unchallenged. At present the difference between haploid and diploid sexes must be referred to the same type of determination as that responsible for the larger size, rougher texture of eyes and other slight changes that distinguish the 3N from the 2N individual.

But outside of the cases like that of the bee, it seems probable that the ratio type of interaction is the general mode. In evidence of this may be cited the vast array of monoecious plants in which the sex relations in the triploid and tetraploid remain the same as in the diploid. Of course there is high sterility in the triploid forms on account of the instability of the 3N group in meiosis, and the consequent production of inviable gametes or zygotes. Among the most striking confirmations of this ratio view of genic balance is seen in the mosses, through the brilliant work of the Marchals, Schweitzer and von Wettstein. For example, they find in a moss with separate sexes that a 2N gametophyte that combines 2 female groups of chromosomes is a pure female, like the haploid female plant (Table II). Likewise the 2N gametophyte that combines two male groups of chromosomes is a pure male plant, like the haploid male plant. But a 2N gameto-

TABLE II

SEX TYPES IN A DIOECIOUS MOSS

Sex Type		X (100)	X′ (50)	A (80)	Ratio $(X+X'):A$	Sex Index
Female	2N	2	...	2	200: 160	1.25
	N	1	...	1	100: 80	1.25
Proterogynous Herm. 3N		2	1	3	250: 240	1.04
Protandrous Herm.	4N	2	2	4	300: 320	.94
	2N	1	1	2	150: 160	.94
[2]Protandrous Herm. 3N		1	2	3	200: 240	.83
Male	2N	...	2	2	100: 160	.63
	N	...	1	1	50: 80	.63

phyte that combines a male and a female group is no longer a single-sexed plant but is a protandrous hermaphrodite. Furthermore, the 4N gametophyte that combines 2 male chromosome groups with 2 female groups (FFMM) is a hermaphrodite like the FM hermaphrodite. But a triploid form in which two groups are female and one is male is a hermaphrodite that is strongly protogynic instead of protandrous. On the other hand, in working with monoecious mosses where the haploid group is a hermaphrodite, then all haploid, diploid, triploid and tetraploid plants were hermaphrodite without distinction, as they should be from their possession of the same ratio of female to male determiners.

A series of sex-indices, similar to those for Drosophila, can be fitted to the dioecious mosses, as shown in Table II. Here it is assumed that there is a pair of chromosomes, X and X′, whose difference accounts for the difference between the female and male types. It is assumed that in both these sexes the net effect of the other chromosomes, that may be represented by A, is male-determining. Then, since the X,A type is a female, the value for A must be less than that of X, *e.g.*, $X=100$ and $A=80$. Likewise, since X′,A is a male, the value X′ must be less than that of A, *e.g.*, $X'=50$. Also, since the FM plant is a hermaphrodite that resembles the normal male more than the normal female, $X+X' < 2A$. And, since the FFM plant is a hermaphrodite that resembles the normal female more closely, $X+X+X' > 3A$. We have thus five limiting equations for the three values X, X′ and A. As the table shows, the assigned values of 100, 50 and 80 are possible, although there may be other slightly different values that would give a set of indices whose intervals would correspond even more closely to the observed differences than do those given.

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[2] This type is not reported as realized.

The same conformity to the ratio rule seems to be true in the haploid, diploid, triploid and tetraploid daturas, as far as I can gather. But on the genic balance view each of the twelve kinds of chromosomes of Datura might have a distinctive internal unbalance of the sex-controlling genes, similar to the unbalance in the fourth chromosome of Drosophila. In Datura there is a full series of forms that differ from the 2N by the addition of a particular extra chromosome. If any of these twelve kinds of chromosomes contain more effective male tendency genes than female tendency genes, or vice versa, then one may well expect to discover that some of Blakeslee's "Apostles" and "Acolytes" have atypical sex-relations.

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SEX AND THE PARTHENOGENETIC-BISEXUAL CYCLE

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AMONG the groups of animals which reproduce both parthenogenetically and by fertilized eggs, there are two general types. In animals of one type there is but one kind of egg, capable of being fertilized, but capable also of developing without fertilization. Animals of the second type produce two kinds of egg, one kind capable only of parthenogenetic development, the other either requiring fertilization or being capable of being fertilized. It is only in the latter of these two types that the reproductive changes may be described as cyclical. Cycles involving two types of eggs, of the sorts just described, are found chiefly in the rotifers, cladocera and aphids. It is to the problems of sex in these groups that your attention is invited.

The cycle of reproduction in the rotifers is more definite and better understood than in either of the other groups. From the fertilized egg of *Hydatina senta,* a rotifer whose cycle is well known, hatches a female whose offspring, parthenogenetically produced, are likewise females. These females of the second generation may all be, like their mother, capable of producing only obligatorily parthenogenetic eggs which develop into females; or some of them may produce smaller eggs which are facultatively parthenogenetic, yielding males if unfertilized, but females if fertilized. There are thus two kinds of female, wholly distinct from one another though alike in gross appearance, which have been variously named male-producers and female-producers, or sexual and parthenogenetic females, or recently, by Storch (1924), mictic and amictic females. All these names are objectionable in that the terms applied to the male-producing type refer to but one of the two possible destinies of its eggs. These two types of female may occur together in the second generation, that is, among the daughters of the female hatching from a fertilized egg, and in any generation thereafter as long as parthenogenetic reproduction is maintained. The facultatively parthenogenetic or mictic females are usually in the minority, but their relative numbers vary within wide limits. Most of the investigations of the relation of environment to mode of reproduction in rotifers have been concerned with the varying proportions of the mictic females.

The cycle of the aphids is similar to that of the rotifers, except that the mode of reproduction is less intimately related, as is pointed out later, to the sex of the individuals. In *Macrosiphum solanifolii*, a typical species, the exclusive mode of reproduction during the summer is parthenogenesis, the parthenogenetic females being either winged or wingless. In warm regions parthenogenesis may continue during the entire year, but in colder climates sexual females and males appear in late summer or autumn. The sexual females are recognizably different from the parthenogenetic ones, and their eggs must be fertilized in order to be laid or to develop. Similarly, in most other species, a female is exclusively parthenogenetic or wholly sexual, but in rare cases both kinds of eggs have been produced by the same female.

The cycle of the cladocera is not unlike those of rotifers and aphids, but the types of female are less sharply defined. Indeed, in *Simocephalus vetulus*, which has been extensively used in experimental work, there is only one type of female. In this species, according to Green (1919), any female after the stem mother, which hatches from a fertilized egg, may produce either ephippia enclosing the sexual eggs, or, by parthenogenesis, males or females, the latter of which again have all three of these possibilities. One female often produces two of these kinds of offspring, and sometimes produces all three. If a female produces only one kind of offspring, this kind is almost invariably the parthenogenetic female.

It is perhaps owing only to the liberal conception and wording of the subject of this symposium that a discussion of sex in the groups exhibiting cycles like these is permitted a place. To many biologists the problems raised by the rotifers, cladocera and aphids, or at least the problems whose solution has been most assiduously sought in those groups, have seemed to be those only of sex production, not sex determination, notwithstanding that the term sex determination has been applied to all of them repeatedly. If, before the end of this discussion, I shall have tried to indicate that the ancient practice of referring to the factors which bring on the generations of males and sexual females, in the groups named, as factors of sex determination, was not entirely incorrect, it will not be because the phenomena then commonly referred to should, in my opinion, be regarded as sex-determining. In the rotifers, the entrance of the bisexual phase of the cycle was made known by the appearance of males, where all had been females before. Though it is now well known that the females are of two kinds and that both kinds must be produced before males appear, that was not patent at first, since all females looked alike. In the aphids and cladocera the sexual females in many species closely resemble the parthenogenetic females, and may indeed sometimes be the same individuals. In these groups also the first readily recognizable sign of the sexual forms was the occurrence of males. It is not surprising that, before the nature of the cycle was well understood, the causation of the sexual phase should have been regarded as sex determination. Whether it should be surprising that the name remained, after the phenomena were shown to be different, depends on one's opinion of human nature.

Some justification for the name sex determination remains, however, even when sex production is kept in the foreground. In the rotifer *Hydatina,* for example, there is a definitely fixed relation between sex production and the determination of one of the sexes. Thus, the male is invariably the offspring of the sexual female. The only way to determine the sex of a male in *Hydatina* is first to determine a sexual female and then wait a generation. Male determination is not, it is true, necessarily final, for the sexual female may be impregnated when young, whereupon the same eggs produce female offspring. Reversal of male determination does not, of course, deprive the first event of its determinative significance. Indeed, if production of a sexual female is not determination of the male in the following generation, in *Hydatina,* then there is no male determination in this rotifer.

There are indications that in the aphids also sex production carries with it certain elements of sex determination. It has been shown (Shull, 1918b) in some lines of the potato aphid, *Macrosiphum solanifolii,* that in the summer winged individuals appear during successive parthenogenetic generations with increasing frequency. The cause of this gradual change, when it occurs, has not been ascertained, but judging from experimental work on wing production in other aphids it is probably due largely to environmental factors. When, in the experiments referred to, the sexual forms appeared in the fall, the sexes showed an evident relation to wings in the preceding generation. Most of the males were born of wingless mothers, while the sexual females were produced chiefly by winged females. There was no rigid relation between these forms, since the same wingless females which produced males also frequently or occasionally produced winged or wingless parthenogenetic females or sexual females as well; and the winged females which gave birth to sexual females also yielded winged and wingless parthenogenetic females and, rarely, males. Notwithstanding these varied capacities, it was nevertheless true that the wingless parthenogenetic females, if they produced sexual forms at all, produced chiefly males, while the winged parthenogenetic females gave birth mostly to sexual females. A like dependence upon wings or their absence has been observed repeatedly in other aphids and is perhaps a general condition. Indeed, systematic writers are accustomed to distinguish separate kinds of parthenogenetic females whose function is the production of the sexual forms, and to apply distinct names to them. Exceptions are recorded by some of these writers, however, and it appears likely that in aphids in general a cycle like that of *Macrosiphum* is common; that is, that there is no type of individual rigidly distinct, even in a physiological sense, as the mother of either of the sexual forms, but that sexual females are produced predominantly by winged mothers, males by wingless mothers. Nevertheless, the cycles of aphids are extremely varied (Patch, 1920).

Under the circumstances described for *Macrosiphum solanifolii,* any condition which brings on the sexual phase while the wingless females are in the majority favors the production of males, while if the sexual phase is delayed until winged females are predominant, females are favored. A probable result of these alternative possibilities is found in the nature of the sexual phase of the potato aphid in our experiments in two recent years. Several years ago, the sexual phase began in the first week of September, males appearing first and in considerable numbers. In the fall of 1924, however, sexual forms did not appear until the middle of October, and males were rare. Even if the gradual increase in the number of winged parthenogenetic females which was observed in our experiments, particularly in one season, should prove not to be a regular occurrence, as may easily be the case if the wing production in this species is environmentally influenced, the time of incidence of the sexual forms would still be highly significant in determining the relative frequency of sexual females and males. Appearance of the sexual forms during a period of few wings would favor males, while abundance of winged females at that time would increase the proportion of sexual females.

The physiological differences between the several types of individual in this species are under investigation, and it is too early to arrive at a conclusion, but it seems at present not unlikely that one or more of the fundamental differences between winged and wingless females are of the same order as those that distinguish the sexual from the parthenogenetic forms and males from sexual females.

Enough has been said regarding the cycle of the rotifers and plant lice to show that, although the expressions "sex production" and "sex determination" properly designate two different phenomena, they are more or less closely related to one another. It is not unlikely that some elements of the two may be identical. That a similar relation exists in the cladocera is not improbable. Varying degrees of intersexuality, affecting both the primary and secondary characters, described by Banta (1918, 1921) and La Vaulx (1919), and alternations between parthenogenetic and bisexual reproduction are both readily effected by environmental conditions; and according to La Vaulx (1922) intersexuality may be induced by at least one environmental factor which several investigators have observed to affect the mode of reproduction.

The form of the cycle, by which is meant the frequency, duration and magnitude of the periods of sex production, is subject to great fluctuation in all these groups. There are inherited differences between parthenogenetic lines, some of which produce few sexual forms, others many, under the same conditions. Within a given line also great modification can be effected by artificial means. In the rotifers, sexual reproduction may be postponed or even prevented by a variety of chemical substances in the medium; or it can be hastened and increased by the use of green protozoan food or, according to our results (Shull and Ladoff, 1916; Shull, 1918a) which Whitney (1917, 1919) was unable to confirm, by increasing the oxygen content of the water. In cladocera, temperature, food and excretions in the medium have been held responsible, either directly or indirectly, for similar advancement or postponement of the sexual phase. In aphids, an old conjecture that low temperature brings on the sexual phases has been confirmed in a sense by several experimenters, in whose minds there lurks, however, a suspicion that the effect of the low temperature is due to its action on the food of the insects. Length of the period of daylight has likewise been thought (Marcovitch, 1923; Davidson, 1924) to influence the cycle, though obviously this may only indirectly influence the aphids through their nutrition. The agents used in regulating the appearance of the sexual forms have been so varied, especially in the rotifers, that no characteristic common to them can be discovered. Their effect must be conceived, therefore, either as a general modification of metabolism, or as being exerted upon some fundamental mechanism which is readily disturbed by a variety of means.

That there is a relation between metabolism and the production of the sexes in *Hydatina senta* has been shown repeatedly in experiments. Periods of abundant sexual reproduction tend to alternate with periods of almost exclusively parthenogenetic forms (Shull, 1915), an alternation which has been mentioned at least by implication by several authors and probably witnessed by all investigators of this species. Periods of abundant sexual forms have been invariably characterized by rapid growth, rapid reproduction, large families and relative indifference to the amount of care bestowed by the experimenter upon the rearing of them. Signs of this increased metabolic activity precede the appearance of the sexual forms, indicating that, if the two events stand in the relation of cause and effect, the increased metabolism is the cause, and that, if both are effects of the same cause, increase in the rate of metabolism results more rapidly than does sex production. This observation has been made, not only by experimental workers, but by those whose interest is systematic or in the field of freshwater biology.

Wesenberg-Lund (1923), for example, in a search for males in numerous species used this method of determining when to look for them. He states that as early as 1898 he had shown that "before a sexual period sets in the species in a given locality will very often increase enormously in number. This observation has been corroborated by almost all later authors."

A striking parallel to the periodical occurrence of sexual reproduction is found in *Paramecium,* as described by Hopkins (1921). Clones of this protozoon differ from one another in their readiness to conjugate. All of them exhibit a periodicity of conjugation, with fairly constant intervals between their periods, but different intervals in different clones. Conjugation is initiated by a period of unregulated division, and it is thought possible that whatever effect external conditions have on conjugation is exerted first on the rate of fission. Practically every point in this statement concerning *Paramecium* duplicates, with slight verbal changes, the corresponding statement for *Hydatina,* indicating that the metabolic relations of sexual reproduction are wider than the parthenogenetic-bisexual cycle.

What is the explanation of the phenomena so characteristic of the cycles in the rotifers, aphids and cladocera —the different types of females, the changes in the mode of reproduction, the increased physiological activity preceding the production of the sexes? Old-fashioned geneticists, among whom I count myself, when seeking explanations of sex and related phenomena, think early of chromosomes. They are entirely free to consider chromosomal changes. since the annoying and disconcerting discovery of one botanist that sex may be explained without chromosomes was probably conceived to relate only to plants. Unfortunately for any chromosome explanation of the cycles in question, the facts so far known regarding numbers speak against it. The first definite chromosome count in the rotifer *Hydatina senta* (Shull, 1921) set the diploid number at 12, alike in parthenogenetic and sexual females. Though reduction occurs in the sexual egg, this event is much too late to have any determinative significance. Tauson (1924) finds in *Asplanchna intermedia* that the diploid chromosome number is 24 (according to Whitney, 1924, it is 52), alike again in parthenogenetic and sexual female. *Asplanchna priodonta,* according to Storch (1924), has 16 chromosomes, the same in both types of female. In aphids, the parthenogenetic females which produce the sexes are like each other and like other parthenogenetic females with respect to chromosome number; and though males and females differ in the chromosome numbers through a type of non-disjunction, the decisive event occurs in an indeterminate proportion of eggs from one mother, dependent presumably, not upon chance, but upon some unknown factor perhaps environmental. In daphnids, likewise, the chromosome numbers appear to be the same in all types of female. Any chromosome difference which may conceivably be responsible for changes in the life cycle of these groups must therefore be invisible ones, and, in the aphids and cladocera, must be more or less easily reversible.

In the absence of a difference in the number of chromosomes between parthenogenetic and sexual females in rotifers, Storch attempted to push back the distinction to an early stage of development. He found that in *Asplanchna priodonta* the chromosomes in the early oocytes of a sexual female undergo synapsis and form tetrads, while the oocyte nuclei of the parthenogenetic female remain in a resting condition during the corresponding stages. These differences were to have been expected. He also found in the early oocytes of the parthenogenetic females certain highly refractive chromatic bodies adhering to the outer surface of the nuclear membrane, which were uncommon or lacking in the sexual females. Storch conceives these bodies to be of importance, perhaps organ-forming substances prepared in the nucleus and conveyed to the cytoplasm. It would be easy to erect about these "Membrankörper" an imposing edifice of theory. Those by whom mitochondria are held in high esteem will no doubt gather these new objects into their fold, especially if they should prove to be important. I refrain from suggesting the inhibiting or stimulating influences which they could be imagined to have upon various physiological processes, for these suggestions will come in due time—and if not, so much the better. To me, the "Membrankörper" appear rather to be signs of a physiological difference not unconnected with the greater growth of the oocyte in whose early stages the bodies are present.

The importance of at least one of these early distinctions between parthenogenetic and sexual females in *Asplanchna* is overestimated by Storch when in his preliminary account (Storch, 1923) he points out that whatever factors convert a parthenogenetic female into a sexual one directly influence the chromosomes so as to bring about synapsis—a statement which ignores the discovery that in *Hydatina* the distinction between sexual and parthenogenetic females dates from a much earlier stage. The decisive event in *Hydatina* occurs in the last two or three hours of the maturation of the parthenogenetic egg. Influences known to be capable of converting a sexual female into a parthenogenetic one are ineffective before and after that time. If *Asplanchna* resembles *Hydatina* in these respects, when an egg is laid the nature of the female which will hatch from it has already been fixed. The synapsis of the chromosomes and the formation of the highly refractive membrane-bodies in the oocytes are but results of determining events which occurred much earlier. They may be used as recognition marks, or as steps by which the different reproductive processes of the adult are attained, but nothing more.

Failure of visible morphological entities to throw light upon the nature of the cycle in rotifers, aphids and daphnians has driven some biologists to look for the necessary determinations in the invisible phenomena of metabolism, as is done also for the sexes of other animals, thus reviving a theory of an earlier age. Proponents of the theory referred to, pointing out that males and their spermatozoa are more active than females and their eggs, hold that anything which increases the rate of metabolism (by which is commonly meant rate of oxidation) tends to the production of males. As applied to the cycles which we are discussing, the theory would doubtless require that anything which increases the rate of metabolism tends to bring on the sexual phase, since in rotifers that is the only male-determination that exists. There are many facts that can be adduced in support of this view. As stated above, the sexual phase is regularly preceded and accompanied in rotifers by rapid growth, rapid reproduction, and large families, although Banta and Brown (1924) have found in cladocera that males are produced by a moderate reduction of the metabolic rate of the mother. The fact that the sexual phase in rotifers is hastened or rendered more pronounced by green food and probably by oxygen lends further support to the theory. Most of the artificial agents which have been used to modify the cycles of aphids and cladocera can be interpreted to act in like manner. What should be our attitude toward this theory?

Probably all biologists recognize that male animals are physiologically different from females of the same species. Probably all will accept the view that differentiation of the sexes during embryonic development is conditioned by differences in the metabolism of the two kinds of embryos. If it is asserted that these physiological differences may result from chromosome differences, there will be few dissenters. The statement that only chromosomes can account for such metabolic differences would be denied by some, perhaps by many. Announcement of the discovery of the particular metabolic difference in the embryos which causes the differentiation of the sexes is met with some skepticism. Even after it is shown that the oxidative processes of one sex are greater, or the storage processes less, than those of the other, it has not been shown that any means whatsoever of altering the rates of these processes will alter sex differentiation. That artificial alteration of the rates of these phases of metabolism may alter the developmental processes in the manner required to distinguish the sexes remains an assumption with but slight foundation. Oxidation and storage are late enough in the chain of chemical events making up metabolism to permit, or even to create, the hypothesis that they are antedated by something else, and that they may be results rather than causes. The hope that a secondary means of increasing oxidation, without going back to its natural cause, would nevertheless alter sex is, in my opinion, as futile as the belief that the speed of lumber-production in a logging camp, though ordinarily measured by the number of trees that fall, could be as effectively increased by a tornado passing through the forest as by sharpening the cutters' tools or by stimulating the laborers to more vigorous operations. If, as is not inconceivable, the changes from parthenogenesis to bisexual reproduction and back again are closely allied to the determination of sex, and if it is sought to apply to these cycles the theory of levels of metabolism, the same objections must be urged against regarding mere rate of metabolism as the cause.

The expression "metabolic level" applied to the causation of sex or of cyclical changes in the mode of reproduction may be well enough chosen if the difference between maleness and femaleness, or between parthenogenesis and bisexual reproduction, is purely quantitative, even if it should prove that oxidation and storage are not the phenomena whose level is at issue. But is the difference purely quantitative? Intersexuality and such phenomena as the graded series of pigeon eggs obtained by Riddle, the varying extent of periods of sexual reproduction in cladocera, and the probable gradual change in aphids leading to the sexual period, seem to speak in favor of a quantitative explanation. If these phenomena are quantitatively determined, what then is to be said of the very sharp and definite differentiation in rotifers between the male-producing and female-producing types of female, which occurs almost momentarily in the maturation of the eggs from which these females develop? There are no intersexes in *Hydatina,* and no hesitation, so to speak, whether a male-producer or a female-producer shall develop. *Hydatina* is equally unequivocal in other matters; witness the considerable constancy in the number of cells in the adult body. There are other animals whose sex phenomena are as little subject to irregularity as are those of rotifers; but in *Hydatina* this decisive determination occurs in the midst of flux and response to the environment as great as that of any other organisms. The two conditions can be reconciled, it seems to me, only by assuming that the decisive event distinguishing parthenogenetic from sexual females is of a different order from metabolic level, but may be partly controlled by metabolic differences. In *Hydatina* this event is single and for some time irreversible. Animals with intermediate sex phenomena may be brought under the same point of view either by assuming several deciding events, or their reversibility, or both. Differences of this sort between animals appear to me much more likely than that their sex phenomena are essentially unlike.

If there is something more fundamental lying beneath the oxidation and storage levels that distinguish males from females, or sexual reproduction from parthenogenetic, what may this something be? Foolish questions invite, and sometimes receive, foolish answers, but the risk seldom prevents the asking. It should be particularly easy in this instance to provide the foolish answer, because, in our present state of knowledge, any answer will be regarded as foolish by some one. It were the part of wisdom to maintain silence, but playing at wisdom seldom makes progress, and it should be no more reprehensible to formulate hypotheses in public than to do so in the recesses of one's private laboratory. Whatever possible explanations are suggested will be derived chiefly from the evidence afforded by cyclical animals, and will be intended to apply only to them.

It is clear that whatever introduces the sexual phase in the aphids, cladocera and rotifers must be rather readily influenced by the environment. It is equally certain in the case of the rotifers that the determination is a sharply defined event, occurring in a very short time, perhaps momentarily, and maintaining complete irreversibility for a whole generation. From comparatively recent work on the manipulation of chromosomes by environment in other animals, it might seem that the most plausible explanation meeting these requirements would be one involving chromosomes. As pointed out earlier, no difference in the number of chromosomes between parthenogenetic and sexual females has been discovered in any of the groups exhibiting cycles of reproduction. It is conceivable, of course, that such differences may involve only behavior of the chromosomes, not their number, thereby implying a difference in chemical or physical constitution. Such a difference in behavior has been found by Morgan (1909) in certain phylloxera, where chromosome adhesions appear to be associated with the distinction between male-producing and female-producing lines. Adhesion of chromosomes may be due to changes in their viscosity, and our knowledge of viscosity of protoplasm is only in its beginnings. There is a great and rather sudden increase in the viscosity of the oocytes of the rotifers at the beginning of the maturation division, which is very nearly the time of the event which determines which sort of female the egg shall become. Whether the viscosity of the chromosomes is likewise altered at the same time, or whether it may be unequally altered in different oocytes, in response to quality of food or the presence of chemical substances in the water, is unknown.

If chromosome behavior in response to physical or chemical phenomena is not responsible for the changes in the cycle, it is not inconceivable that there is a more direct or more general action of physical or chemical changes. Here a wide field of speculation is opened. When the subtle influence exerted upon higher animals by green or raw vegetable foods is thoroughly understood, it may be possible to conjecture why green protozoan food produces such a remarkable increase in sexual reproduction in rotifers. When it is known why unobstructed sunlight is so effective in stimulating certain phases of metabolism and preventing deficiency diseases, or why mere exposure of otherwise non-stimulating foods to sunlight has a similar effect, we may be nearer an understanding of sex and life cycles. It is a permissible conjecture that these influences are at least as much physical as chemical. It is not necessary to pursue the physical explanation of life processes as far as does Mathews (1924) when he adopts the view that living matter differs from non-living in the magnitude of its electronic orbits and the consequent greater energy involved. One who lingers over Mathews' engaging hypothesis is apt to imagine himself an electron, revolving about a nucleus of truth, though perhaps in ever-widening orbits until the energy involved is positively dangerous. We may perhaps wait until physicists have had an opportunity to express their judgment upon this particular hypothesis. Should they reject it, there is nevertheless room to anticipate, or at least to hope, that the metabolic process at the basis of oxidative levels, sex differentiation and modes of reproduction may nevertheless, if the search must go so far, some day be found to rest upon the ultimate structure of matter and energy.

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SEX DIFFERENTIATION IN THE BULLFROG (RANA CATESBEIANA)

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THE problem of sex determination and differentiation in animals is so complicated, the field so vast and the opinions expressed on the matter so conflicting, it would be impossible in the brief time at my disposal to attempt a comprehensive discussion of this fascinating and difficult subject. Instead I shall confine my remarks to an account of an embryological and cytological study of sex differentiation in one lowly member of the vertebrate group—the bullfrog. In the first portion of this paper the facts of sex differentiation in *Rana catesbeiana* will be presented, the latter part will be devoted to general theoretical considerations.[1]

During the past six years study of *Rana catesbeiana* material from various parts of the United States has shown the existence of local races which differ markedly in regard to the time of occurrence and character of the developmental process by which the definitive testes of the male animals arise. The male larvae of most bullfrog races present an interesting and complex series of cyclical changes in gonad and germ-cell development, which may be lacking in other races inhabiting the same region. In some strains the definitive male gonad is developed very early in larval life and the two sexes are easily distinguished. These are called differentiated races, *i.e.,* there is no question as to the sex of the gonads, even in young tadpoles. Other local races show a peculiar gonadic development, chiefly affecting the males, the definitive testis sometimes not appearing until near the end of the second year of larval life. In the young larvae of such races the sexes can not be distinguished with certainty. Races exhibiting the peculiar development of the male gonads are called undifferentiated, that is to say, the morphological features of the definitive testis are not established until late, and in some races not until two years after the time when the definitive testis has formed in other races.

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[1] In this paper only the writer's views on sex differentiation in anurans have been presented, owing to time and space limitations. Views differing from those presented here will be found in the following references: Hertwig, R., Biologischen Centralblatt, 1912, Bd. 32; Kuschakewitsch, S., Festschr. f. R. Hertwig, 1910, and Witschi, E., Arch. f. Mikr. Anat., 1914, Bd. 85 and 86; also Zeitschr. f. ind. Abst. u.Vererbgsl., Bd. 29.

There is nothing noteworthy about the gonads of the differentiated races; at any time during development the sexes are easily distinguished. The situation is quite otherwise in the undifferentiated strains, for when the gonads of the larvae of such races are examined, say at metamorphosis, they may be grouped into three distinct types, testes, ovaries and a third type of gland not so easy to classify (Fig. 1). It is an elongate, cylindrical body, generally smooth in outline. This type of gonad differs very greatly from both testis and ovary and in some respects is intermediate between the two. The gonad resembles the ovary in regard to length and in the absence of sex cords and rete apparatus, but it resembles the testis in that it is cylindrical, generally smooth and attached to the fat body (Fig. 1, B and C). All gonads of this type ultimately transform into typical testes by a peculiar indirect method; in fact, all male gonads of the so-called undifferentiated strains first develop the peculiar type of gonad shown in B and C, Fig. 1. In some animals even after two years one may find a typical testis

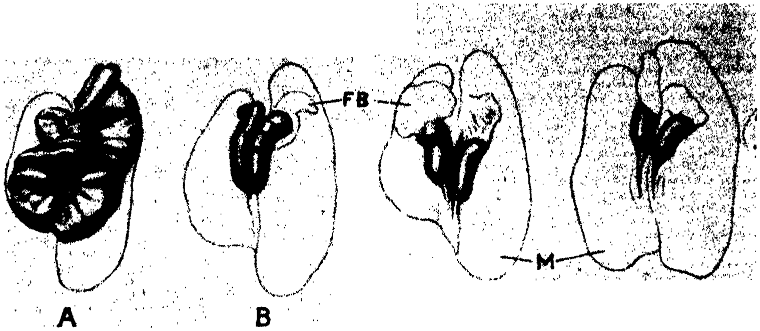

FIGURE 1

A. Ovary of a tadpole approaching metamorphosis; B, progonad of male tadpole of same race and stage of development as A; C, progonad of larva at metamorphosis transforming into the definitive type of testis shown in D. FB, fat body; m. mesonephros.

on one side and the elongated cylindrical gonad on the other (Fig. 2).

According to some investigators the elongated, cylindrical gonads are really ovaries, and all the tadpoles of the undifferentiated races such as here described are said to be females, and later, under the influence of environmental factors, 50 per cent. of the animals transform into males. The 50 per cent. which are thought to undergo such a transformation are those larvae which possess the peculiar gonads. The writer does not regard this cylindrical type of gonad exhibited by males of the undifferentiated races as an ovary, but rather as a sexually neutral germ gland or progonad with neither sex predominating. The germ cells may differentiate as spermatocytes, a few oocyte-like cells may appear or development may proceed only as far as the pachytene stage and then cease. The sex-differentiating substances are evidently present in the larvae in amounts sufficient to counterbalance one another. These progonads ultimately develop into definitive testes by a remarkable transformation process to be described later.

One very interesting point brought out in this study was the fact that differentiated and undifferentiated frog races may inhabit the same region or even the same pool. It is to be understood that the larvae of both types of races are similar in all respects except in regard to gonad development.

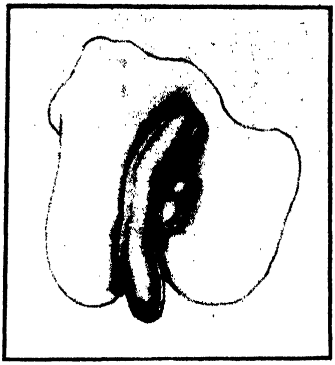

FIGURE 2

Gonads of larva at metamorphosis. At the left is shown the persisting progonad while on the right is the definitive testis. The transformation of the progonad into the definitive testis generally occurs more rapidly on one side than on the other in *Rana catesbeiana.*

But what is the explanation for the existence of differentiated and undifferentiated frog races? Why is it that in differentiated strains the ovaries and testes of the larvae are morphologically differentiated at a very early stage, whereas in undifferentiated races the gonads of the male animals undergo a series of peculiar changes, first developing a sexually neutral progonad, which is finally replaced by the definitive testis. Although the complete solution of the problem is not now at hand, the direction in which it is most likely to be found was clearly pointed out years ago by Professor Richard Hertwig in his interesting and suggestive hybridization experiments with *Rana esculenta,* where he was able to show unequivocally that the gonadic differences between the various local races of Central European frogs are due to differences in the hereditary constitution of the races.

Hertwig found that when males and females of differentiated races are bred, only morphologically sexually differentiated progeny result, and the sex ratio is 50 ♀ ÷ 50 ♂ ; on the other hand, when males and females of undifferentiated races are bred, only larvae showing indirect gonad development arise, or, according to Hertwig, who considered the elongate cylindrical gonad as an ovary, the F_1 generation are all hermaphrodites, in the sense that the animals are regarded as females, 50 per cent. of which later become males, or else sexually indifferent larvae, which were also thought to show first certain female characters. The results of crossing individuals of the differentiated and undifferentiated races gave results of particular interest. One and the same female crossed with different males gave different results, as also did crosses of one and the same male with different females. The following table summarizes several of Hertwig's crosses:

TABLE I

1. $\frac{\text{♀ A} \times \text{a ♂}}{\text{1 ♀} \div \text{64 J} \div \text{3 ♂}}$	2. $\frac{\text{♀ A} \times \text{b ♂}}{\text{69 J} \div \text{54 ♂}}$	3. $\frac{\text{♀ A} \times \text{d ♂}}{\text{50 ♀} + \text{52 ♂}}$
4. $\frac{\text{♀ B} \times \text{♂ a}}{\text{109 J}}$	5. $\frac{\text{♀ B} \times \text{♂ b}}{\text{34 ♀} \div \text{52 ♂}}$	6. $\frac{\text{♀ B} \times \text{d ♂}}{\text{142 ♀} + \text{140 ♂}}$
7. $\frac{\text{♀ L} \times \text{♂ f}}{\text{59 ♂} \div \text{52 ♀} \div \text{2 J}}$	8. $\frac{\text{♀ L} \times \text{♂ 1}}{\text{111 ♂} \div \text{101 ♀}}$	9. $\frac{\text{♀ L} \times \text{♂ d}'}{\text{3 ♂} \div \text{11 ♀} + \text{190J}}$
Undifferentiated Race a and d′	**Differentiated Race b, d, 1, and f**	**J = Sexually indifferent animals, *i.e.*, morphologically sexually undifferentiated. A normal sex ratio becomes established some time after metamorphosis.**

In the table males and females belonging to the same type, *i.e.*, differentiated or undifferentiated races, are denoted by corresponding letters. The table shows that female A produces when bred with a male of its own race practically nothing but indifferent individuals, of which half later will develop the definitive male type of gonad by the indirect method. Consequently, both animals belong to the undifferentiated races. With the male b the same female (A) produces early sexually differentiated males and numerous individuals with sexually indifferent germ glands (denoted by J) which later on become females. Male b in this cross belongs to the differentiated race; when mated with male d, however, the female A produces normally differentiated individuals.

Female B crossed with male d gives the same result as the female A crossed with this male, whereas the result of the cross with male b is quite different from that where female A was crossed with the same male. The results of crossing female L with males F, 1 and d′ give somewhat different results. The hybridization data are not clear, but the essential point here is that the experiments show clearly the zygotic nature of the factors involved in gonad development and differentiation of the two types of frog races.

Embryological study of seven frog races from different parts of the United States shows that the developmental history of the male gonads of differentiated races is much the same, whereas great variation exists among the undifferentiated strains. Practically every race differs in some respects from the others. Thus a race of larvae obtained from near Lawrence, Kansas, differs from any other studied, in the precocity of the maturation cycle of the progonad germ cells. The progonad of immature tadpoles of 60 mm may be filled with spermatocytes, yet the glands exhibit none of the morphological features of the definitive testes and are later replaced by normal male gonads. A race obtained from Douglas County, Kansas, and one from West Haven, Connecticut, resembled one another, but both differ from other races in that the sex cords do not appear until near the end of the second year of larval life and consequently the definitive testis which depends for its development upon the sex cords does not replace the progonad until metamorphosis or after. In most frog strains sex cord formation is not delayed two years. A race found at Seymour, Connecticut, does not even present any morphological indications of sex differentiation until the tadpoles are about eight months of age. The germ glands remain mere undifferentiated clusters of germ cells long after sex differentiation has occurred in other races inhabiting the same region.

Because of these facts, it is necessary, in any attempt to explain the peculiar gonadic condition of frogs, to assume that besides the fundamental zygotic difference in the factors for gonad formation between the two types of frog races, the undifferentiated races vary among themselves slightly with regard to the potency or intensity, say, of the factors concerned with gonad differentiation. Hence, when such races are crossed, and they intermingle freely in nature, all kinds of qualitative and quantitative variations in gonad development and differentiation appear.

However, to say that the cause of the peculiar and variable gonadic conditions exhibited by the local frog races is zygotic in origin does not solve the problem. We are still confronted by the question as to the significance of the germ gland and germ cell changes of the undifferentiated strains. The essential problem facing the student of sexual conditions in tadpoles is the determination, beyond peradventure, of the nature of such glands as those shown in Fig. 1, B and C. Are such gonads male, female, sexually neutral or intersexual, and if the latter, to what degree? Statistical study of the sex ratios and hybridization experiments throw no light upon this problem, and our only recourse is to embryology and cytology. The developmental history of germ cells and germ glands of the various races should give some clue to the solution of the problem. We shall begin with a brief description of the morphologically undifferentiated germ gland common to all races in early larval life.

If the gonads of 25–30 mm tadpoles are examined they will be found to consist of a peripheral ring of large germ cells, enclosing a central cavity filled with web-like connective tissue. The cavity is the primary genital space and the germ cell ring is the germinal epithelium. Segmentally arranged masses of cells from the mesonephros project into the gonad and develop large cavities. These structures are the sex cords and the sex cord cavity is the secondary genital space. The sections through this type of gonad shown in Figs. 3, 4 and 5 are characteristic of all bullfrog tadpoles at an early stage and exhibit the ground plan of gonad structure out of which both ovary and testes are differentiated.

The ovary represents a further development of the structurally indifferent gonad. If such glands as shown in Figs. 3, 4 and 5 become ovaries the sex cords cease growth and the germ cells of the epithelium increase greatly in number. The sex elements arrange themselves in "nests" or clusters surrounded by mesenchymal cells. The cells within the nests or cysts are sister cells and pass through their cyclical changes simultaneously.

The chromosomes of the germ cells of female larvae very early undergo synapsis and the cells enter the growth period, pass through the early stage of pseudoreduction and become oocytes. The germ cell nests or cysts are destroyed by the enlarging cells and only a few persist at the periphery of the gonad (Fig. 6).

The sex cords form in the young ovary just as in the testis but do not develop very far and are abortive structures. They give rise to the rete ovarii and the ovarial sacs.

In contrast to the ovary, the site of testis formation in differentiated strains is shifted from the peripheral germ epithelium to the sex cord region and any tadpole is to be regarded as male whose sex cords show the presence of germ cells. The sex cords of male larvae at the time of sex differentiation are structurally identical with those of the ovary and undifferentiated gonad. However, they show great activity in that cells migrate out from them to the germ cells of the germinal epithelium, thus forming short cross cords with expanded ends (Figs. 7 and

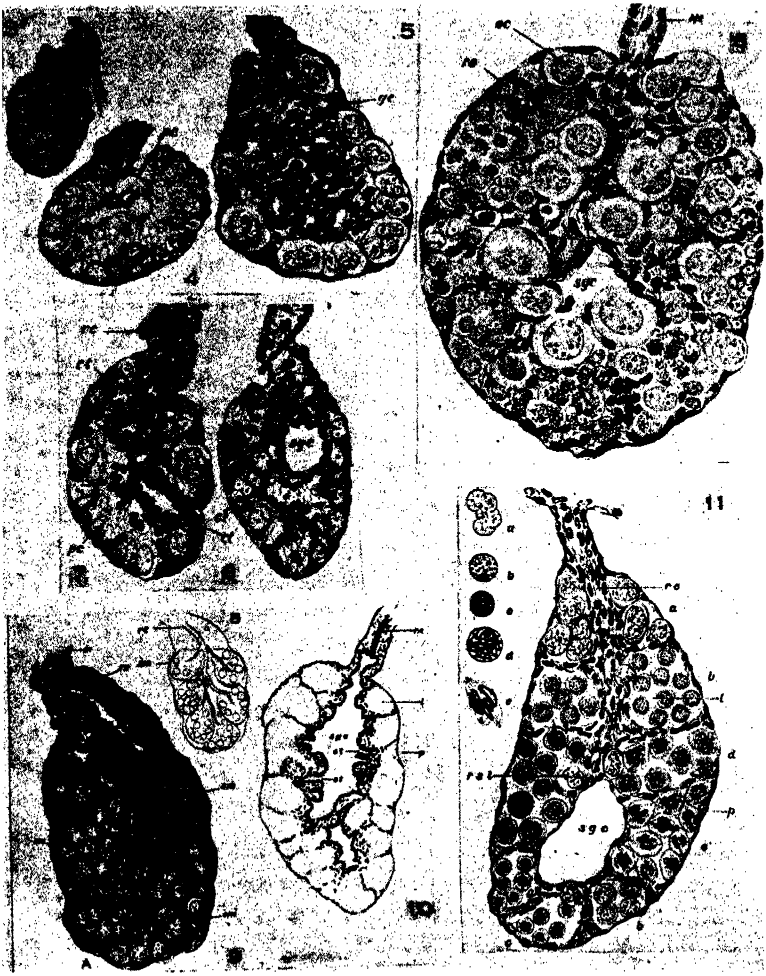

FIGURE 3

Section through the undifferentiated gonad of a 25 mm tadpole. The small cells at the hilus region are migrating sex cord elements.

FIGURE 4

Section through the gonad of a 30 mm tadpole between the segmentally arranged sex cords. pc is the primary genital space.

FIGURE 5

Section through the sex cord region of an undifferentiated gonad at its maximum development. gc, germinal epithelium. The mass of small cells constitute the sex cords.

8, CT). These grow around the sex cells and the germ elements pass into the walls of the sex cords. The latter grow very rapidly and anastomose with one another, forming a core of tissue extending the length of the gonad. All the sex cells pass into the cords in the manner described, leaving the peritoneum empty. After reaching the sex cords the germ cells multiply rapidly and form solid cyst-like structures surrounded by mesenchymal elements. These are the fundaments of the testis ampullae, which later by elongation become the short sperm tubules and connect with the vasa efferentia (Fig. 9, am). The essential features of testis differentiation are established with the appearance of the ampullae, and we need not concern ourselves here with the later development.

The early stages of gonad differentiation in the future males of the undifferentiated races are similar to the ini-

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FIGURE 6

Section through a young ovary. Sgc is secondary genital cavity (sex cord cavity); oc, oocyte; ro, residual oogonium. Note that the sex cords of the undifferentiated gonad (Fig. 5) play no rôle in ovarian development.

FIGURES 7 AND 8

Sections through gonads showing the first signs of differentiation into testes. At CT are cords of cells from sex cords passing out to germ cells in the epithelium and the latter passing into the cords. Sgc, secondary genital space; pc, primary genital space.

FIGURE 9

Section through young testis. The germ cells have passed from the epithelium into the sex cords and are organized into clusters surrounded by mesenchymal elements. The definitive sex cords or rete cords, rc, have formed and are connecting up by short tubules with the germ cell clusters, or ampullae, am.

FIGURE 10

Section through the progonad of larvae of an undifferentiated race. Animal approaching metamorphosis. Note the greatly thickened walls of the gland divided into lobules, 1; the definitive sex cords, rc, are just appearing after two years. Cross tubules, ct, are growing out to the lobules.

FIGURE 11

Section through the progonad of a 60 mm larva before the definitive sex cords have formed. Note the lobules filled with spermatocytes and the migrating sex cord cells at rc. The residual spermatogonia are shown at rsl. A–e, indicate the type of maturation cells present.

tial steps in ovarian formation (Fig. 11). The single layer of germ cells in the epithelium thickens by several strata and the cells become arranged into clusters, each group surrounded by a mesenchymal covering. The sex cells, with the exception of one or two in each cyst, enter pseudoreduction, just as do those of the ovary, and pass through the early maturation stages. At this stage of development the gonad resembles the young ovary at the beginning of differentiation. Both are sacs of germ cells, the latter arranged into nests or cysts forming the thickened wall (so-called germinal epithelium) of the gonad surrounding a central cavity. However, there is one very important difference between germ-cell development of the ovary and that of the progonad of the males of the undifferentiated races. In the ovary the germ cell cysts are soon obliterated by growth of the oocytes (Fig. 6), whereas in the males of the undifferentiated races (Fig. 11) these cysts form the anlagen of sac-like lobules radially arranged about the central sex cord cavity which persist and form the progonad. Moreover, the germ cells of the ovary soon enter the second growth period, becoming typical ova. Conversely, in males of the undifferentiated races the progonad germ cells only rarely assume an oocyte-like character, but instead become spermatocytes or degenerate at some stage of the maturation cycle in prespermatogenesis. Compare Figs. 6 and 11.

The germ cells begin maturating soon after the formation of the lobules, and all stages of the germinal cycle up to the anaphase of the heterotypic division are encountered (Figs. 11 and 17–34). Oddly enough, the first maturation division is never completed. The spindle apparatus breaks down owing to fragmentation of the centrosome, each fragment sends out fine filaments which attach themselves to the tetrads and the latter are literally pulled to pieces. Sometimes giant spermatid-like structures are formed by growth of an axial fiber from the centrosome of a degenerating spermatocyte (Fig. 26). In some progonads, along with the abortive spermatocytes, are large oocyte-like cells, and it is not at all uncommon to see the two types of cell side by side within the same lobule. Many gonads do not show the oocyte-like bodies, whereas in others they are fairly numerous (Fig. 30).

The condition just described where the progonad of the future males of the undifferentiated races consists of greatly thickened walls divided into numerous lobules, the cell contents of which are undergoing maturation and degeneration, persists in some races for two years; in others, for a much shorter period (Figs. 10 and 11). During this time no indications of definitive sex cords may appear, and consequently such progonads lack rete apparatus and efferent ducts. The appearance of the sex cords is sudden and marks the beginning of typical testis formation, for with the development of the cords the entire morphological character of the progonad undergoes a radical transformation.

The sex cords form from the extragenital sex cord cells just outside of the gland, as open tubules extending from the hilus, deep into the gonad, giving off side tubules which grow out to the lobules of the progonad which until this time were mere blind sacs of maturation cells (Fig. 10, rc and ct). These cross tubules from the main sex cord abut directly against the residual spermatogonia of each lobule, which have persisted unchanged through the maturation cycle (Fig. 11, rsl). Cells from the tubules then grow around these spermatogonia, cutting them off from the lobules, and they now pass into the sex cords (Fig. 12). Generally one residual cell is left in each lobule (Fig. 13, rsl). As development proceeds the sex cord walls become filled with the residual spermatogonia which have migrated down the cross tubules, and the ampullae of the definitive testes start developing. These structures arise in a peculiar manner: small areas of the sex cord wall, bearing one or more of the spermatogonia, bulge out into the sex cord cavity and growing from the base region are pushed across the cavity to meet and fuse

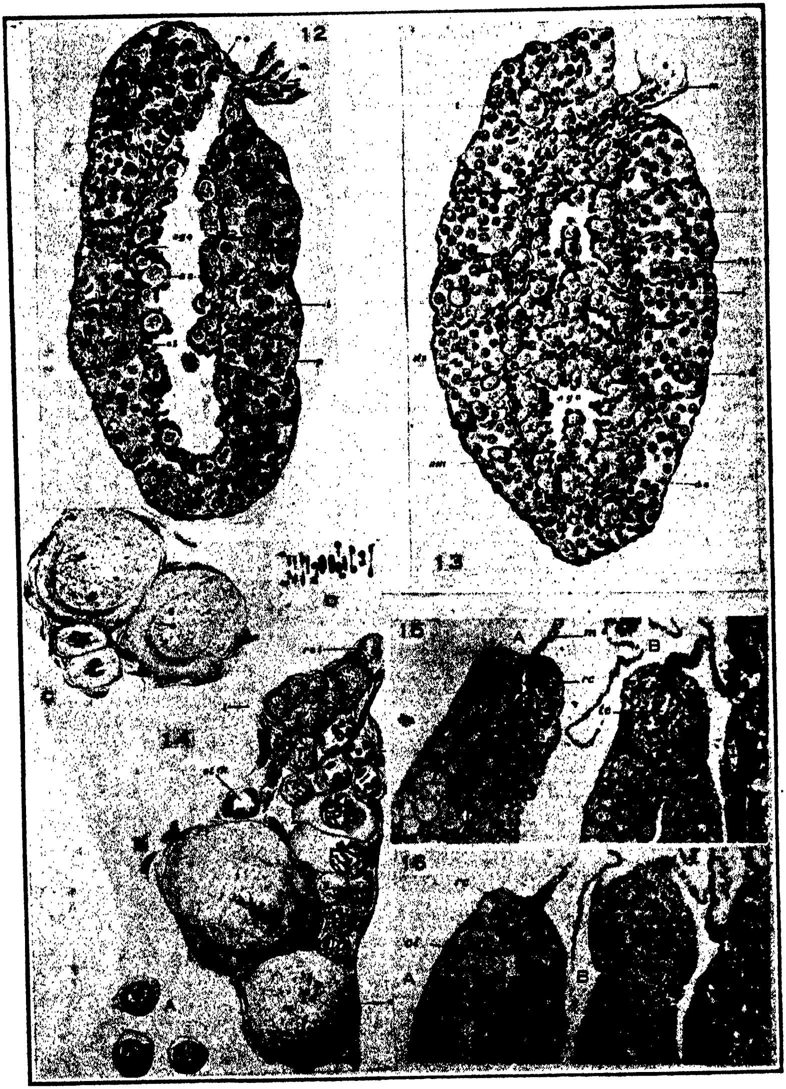

FIGURE 12

Section through the progonad of a larva showing the first stages in the formation of the definitive testis. The residual spermatogonia of the lobules have passed down the cross tubules, ct, into the sex cords, rc.

FIGURE 13

Advanced stage in formation of the definitive testis. The germ cells in the sex cords have multiplied and are arranged into clusters, the testis with the opposite wall. The cavity (see Fig. 12) is eventually cut into very small compartments in this way, each surrounded by a ring of spermatogonia and representing the fundament of an ampulla or sperm tubule (Fig. 13, am).

The gonads at this stage show an outer husk or rind of progonad lobules filled with degenerating maturation cells and a central core of spermatogonia arranged into testis ampullae connected by tubules with the segmentally arranged vasa efferentia (Fig. 13). Eventually all the maturation cells of the progonad lobules degenerate, the lobule being slowly refilled with secondary spermatogonia arising from the residual cells remaining within the lobules. Such lobules may be directly converted into definitive testis ampullae, or they may be broken up and formed anew. There is some variation among the races in regard to this point.

Almost immediately upon completion of the definitive testis, a second prespermatogenic cycle occurs, but this time morphologically normal spermatozoa are produced.

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ampullae, am. Note the outer rind of progonad lobules filled with maturation cells. Rsl, residual spermatogonia in lobule; ds, degenerating spermatocyte; nh, degenerating cells showing oviform hypertrophy—these cells are not oocytes.

FIGURE 14

A. Typical first spermatocyte prophase tetrads from gonad shown in figure 16A; B, enlarged drawing of a portion of the gonad shown in figure 16A. Note spermatocytes and oocyte-like cells side by side; C shows spermatocyte division figures together with oocyte-like cells; D, the tetrads shown on the spindle in C. All these drawings are from the gonad shown in figure 16A. Rsl, residual cell; 1, lobule; CT (?), probably the cut end of a cross tubule.

FIGURE 15

Either an ovary, or an organ of Bidder, transforming into a testis. A and B are gonads of the same animal; rc, sex cord; te, testis forming.

FIGURE 16

Sections through the same gonad as shown in figure 15. One gonad, A, shows first appearance of the sex cords and cross-tubles, ct; the other, B, shows the transformation of the hilus (sex cord) region into a testis.

It is to be emphasized here that the maturation cycle occurring in the progonad is abortive, mature sex products are never produced, and it is peculiar in other respects. The size of the germ cells and chromosomes is much greater than that characteristic of the maturation cycle of the definitive testis. Probably the most striking difference is the type of tetrad formed. The bivalent chromosomes of the progonad are strikingly similar to those of urodeles, being large open rings, whereas the bivalents of the definitive testis spermatocytes are small thick rings or dumb-bell-shaped bodies. I have never seen tetrads in adult frogs which resembled those of the larval progonad.

It is obvious that the process we have just described involves the formation of two gonads in males of the undifferentiated races, a progonad and a definitive testis, and that the germinal elements of the latter arise from the residual spermatogonia of the former (Figs. 12 and 13). The character of the progonad is not uniform, however, for in almost every undifferentiated race it differs in some way in regard to the degree of development and differentiation attained before being replaced by the definitive testis. It is highly developed in some races and persists for two years without any indication of retrogressive changes. In other strains it is replaced by the definitive testis early in larval life, and even in the same local race much individual variation occurs. The same thing is true with regard to the degree of sex differentiation exhibited by the progonad. In some races, notably one found at Seymour, Connecticut, the gonad is totally undifferentiated and exists as a mere cluster of indifferent germ cells until the animals are approximately eight months of age. In other strains, the progonad is filled with spermatocytes, and insofar as the cell cycle is concerned is male, but the gonad has none of the structural features of a frog testis and is destined to degenerate and be replaced by a definitive testis. Figure 11 is of this type of gland. On the other hand, the progonad of some races may exhibit spermatocytes and oocyte-like cells side by side, or neither may appear, and only germ cells in early pseudo-reduction stages and residual spermatogonia are found.

The belief was expressed early in this paper that the peculiar gonadic cycle characteristic of the males of the undifferentiated frog strains is zygotic in origin, and that the minor variations in gonad development and differentiation occurring among the local undifferentiated frog races was due to hybridization of local races, each probably differing from others by minute quantitative variations in the amount of sex differentiating substances distributed by the chromosome mechanism at fertilization. However, the question of the significance of the germ gland and germ cell changes, from the standpoint of sex differentiation, was left unanswered pending the results of embryological and cytological study.

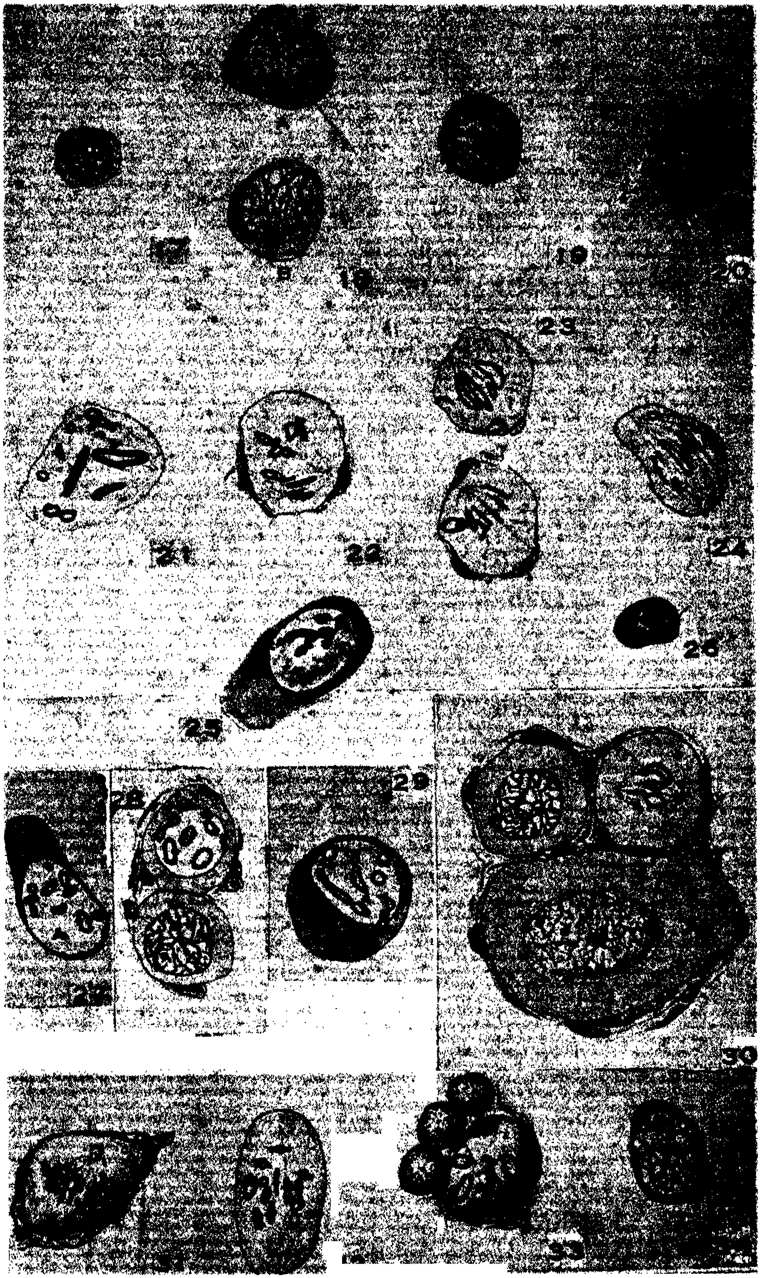
If we accept the view prevalent in some quarters that the elongate, cylindrical progonad is really an ovary, then the problem would be simplified and we could say that we were possibly dealing with a case of zygotic intersexuality comparable to that described by Goldschmidt for the gypsy moth. However, it is evident that the progonad is not an ovary; in fact, it differs from an ovary in several well-marked features (Fig. 11). The progonad germ cells appear to be sexually neutral and capable of differentiating either into oocyte-like cells or spermatocytes, or of remaining for long periods in the pachytene stage and finally disintegrating without developing either way. But no matter which line of sex differentiation the majority of the germ cells follow, the entire germ gland undergoes degeneration and is replaced by a different type of gonad—the definitive testis.

It is the writer's belief that the first formed germ gland of males of the undifferentiated frog races is really a sort of progonad which is sexually neuter, owing to the balance struck between the male and female sex-differentiating substances distributed to the zygote by the chromosome mechanism. In these races, owing to the balanced condition of the contrary sex-differentiating substances in the larvae, the germ cells of the progonad can differentiate along either male or female lines, depending upon the quantitative relations existing between the sex-differentiating substances within the cells. In bullfrog larvae, in general, the male features predominate so that in many larvae the progonad can legitimately be called a protestis (Fig. 11).

The development of the progonad into a testis is hardly comparable to the transformation of an ovary into a testis, such as described for intersexual gypsy moths. In these forms the ovary develops in a perfectly normal fashion and ova and egg tubes are typical, then at a certain critical point, the "switch over" reaction occurs and the ovary disintegrates and a testis forms. However, the progonad of the tadpole is far from being an ovary, but is in fact a sexually neutral gonad which may persist for a few months or for two years, and present all kinds of quantitative variations among the local races as to the degree of development attained. The progonad is sexually neutral, not from any lack of male and female sex-differentiating substances, but because they are present in the larvae in practically equal quantities, and the germ gland is the morphological resultant of the balance struck between them.

If the sexual neutrality of the progonad is due to balancing of the contrary sex-differentiating substances resulting from the hereditary factors introduced into the zygote at fertilization, then it is to be expected that cases will occasionally arise where the equilibrium has been temporarily disturbed and one or the other sex gains the ascendency. Such cases have been encountered.

In the mass of material studied, four cases appeared which seemed beyond doubt to represent the actual transformation of an ovary into a testis, the ovary in these cases being progonads which greatly resembled a Bidder's organ such as occurs in Bufo. Microscopically the



Figures 17–34—The maturation cycle of the progonad germ cells.

gonads looked like ovaries or Bidder's organs and were filled with oocyte-like cells (Figs. 15 and 16). At the hilus region, however, definitive male sex cords were present with cross tubules growing out toward the peripherally located residual cells. The oocyte-like cells in the vicinity of the sex cords were degenerating and being replaced by typical testis ampullae and spermatogonia. In the regions between the sex cords the gonads could not be distinguished from true ovaries or Bidder's organs.

Close cytological study of the gonads revealed numerous cysts of prophase spermatocytes and cells at the anaphase of the heterotypic mitosis side by side with apparently typical oocytes (Fig. 14). Such spermatocytes did not appear in the testis portion of the gonad—only spermatogonia were present there—and they differentiated directly from the diplotene cells of the young lobules of the gonad. Their appearance coincided with the appearance of the sex cords. Thus the evidence is very strong that previous to formation of the sex cords the younger maturation cells at the periphery of the gonad developed into oocyte-like cells and not into spermatocytes. The sex cords apparently secreted or were associated with some substance, *i.e.*, enzyme or hormone which destroyed the large oocyte-like cells, stimulated multiplication of the residual cells and induced cells in the early stages of pseudoreduction to develop into spermatocytes. In these few cases the progonad appeared to be predominantly female. To offset such examples of the early dominance of the female sex-differentiating substances, we have numerous examples showing the dominance of male influences on the progonad, where the maturation cells all developed into spermatocytes, the definitive testis not appearing until the sex cords formed some time later (Fig. 11).

Throughout this paper constant reference has been made to the sex cords as highly important factors in gonad development and differentiation. This point can not be too greatly emphasized, for the length of persistence and degree of development attained by the progonad of the various local frog races depends upon the time of appearance of the definitive sex cords. It is greatest in those races where the sex cords do not form until after two years, and least in the strains where the cords develop early in larval life. The fate of the progonad is sealed when the definitive sex cords form, for immediately changes occur which lead to establishment of the definitive testis and destruction of the progonad, no matter whether its germ cells are differentiating as oocyte-like elements or spermatocytes or neither. The sex cords apparently are the morphological expression of the active determining agents for definitive testis development and exert or are associated with some influence which enables them to override any differentiating tendencies the progonad and its germ cells may have exhibited previous to their formation.

It will be recalled that sex cords are formed in the morphologically sexually indifferent gonad of bullfrog larvae of all races (Figs. 3, 4 and 5). In females of differentiated races and in all animals of the undifferentiated strains, the only trace of sex cords the gonads possess are those of the indifferent gland. The sex cords of males of the differentiated races grow rapidly and become very active, sending out side growths which pass around the sex cells of the germ epithelium and literally drag them into the cords, so to speak (Figs. 7 and 8). The second formation of cords, *i.e.*, the active definitive structures of males of the undifferentiated races, does not appear until late; consequently the gonad which continues development in their absence lacks the necessary morphological basis for definitive testis formation and forms a hollow sac of lobules and remains so until the sex cords appear (Figs. 10, 11 and 12). The definitive testes can not develop in the absence of sex cords and the appearance of the latter definitely decides the course of development. Since the sex cords are peculiarly male structures developed under the stimulus of male sex-differentiating substances, and since they so profoundly transform the morphological features of the progonad built up in their absence, it seems not unlikely that they secrete or give rise to some physiological agent, probably of hormonal or enzymic nature, which induces the radical changes leading to definitive testis formation. The time of appearance of the cords in males of undifferentiated races is probably zygotically determined, though what the factors are that delays their formation is unknown.

Up to the time of appearance of the definitive sex cords the progonad is sexually neutral, the germ cells apparently possessing the power to differentiate either into spermatocytes or oocyte-like elements or of remaining in early stages of pseudoreduction and not differentiating either way. Following sex cord formation the sexual neutrality of the progonad no longer exists and the residual spermatogonia of the old gonad become the germinal elements of the new testes—the maturating cells degenerating no matter whether they are oocyte-like elements, first spermatocytes or cells in early pseudoreduction stages showing no differentiation (Figs. 10, 11, 12, 13 and 15).

Our study of sex differentiation in *Rana catesbeiana* leads to the conclusion that in frogs the sex-determining and differentiating factors are distributed by means of the chromosome mechanism, and that there are two opposing sets of factors, one of which (that for maleness, since the male frog is probably heterozygous for sex) is carried elsewhere than in the sex chromosome, probably by the autosomes, as suggested by the work of Bridges on Drosophila. It is necessary to assume that factors for maleness and for femaleness are present in every fertilized egg or for that matter in every germ cell of the resulting individual in a definite quantitative relation, the given sex of both individual and cell being the resultant of the proportional relation existing between these quantitative determiners.

In the differentiated frog races from the beginning of development the quantitative relations of the male and female sex-differentiating substances distributed to the zygote by the chromosomes are such that the sexes are clearly differentiated at an early larval stage. The idea may be graphically represented by modifying the scheme employed by Goldschmidt to explain his intersexual moths.

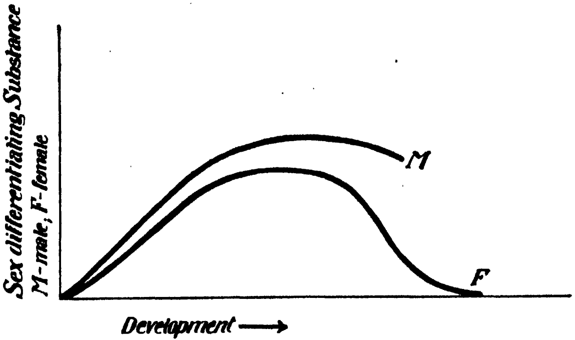


FIG. 35

On the other hand, the quantitative relations of the sex-differentiating substances in males of the undifferentiated races are such in early larval life that the developing gonad is neither a definite testis nor an ovary, but a peculiar sexually neutral structure, combining in a curious fashion the morphological features of both ovary and testis, and from the standpoint of differentiation poised uncertainly between the two sexes but not differentiating definitely into either. The idea may be expressed thus:

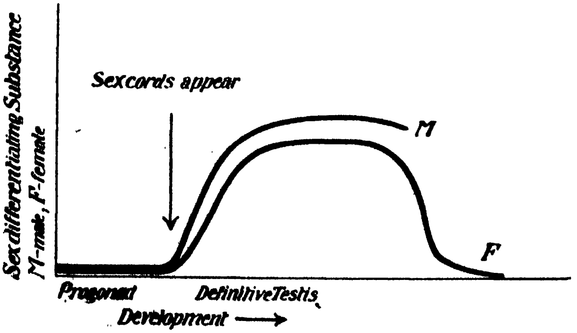


FIG. 36

The balanced condition of the contrary sex-differentiating substances of the progonad is definitely overthrown with the appearance of the sex cords. These structures clearly exert a decisive influence, bringing about degeneration of the progonad and formation of the definitive testis from its residual cells, but the physiological nature of the agents through which the cords work is unknown. It is obvious that the cords themselves are but the morphological expression of deep seated physiological changes occurring in the organism.

Since the sex ratio of adult bullfrogs of all races is 50–50, and the elongate, cylindrical progonads invariably develop into definitive testes, the 50 per cent. of animals of the undifferentiated races possessing these glands are to be regarded as genetic males exhibiting in the course of larval development a transitory, sexually neutral phase. In large numbers of these larvae, however, the progonad is not neutral but is really a protestis in that the germ cell cycle is distinctly male (Fig. 11).

The process by which the progonad is transformed into a definitive testis is not to be regarded as sex reversal (except possibly in a few exceptional cases, and even here it is impossible to know whether one is dealing with a true ovary or a transitory gonadic rudiment comparable to Bidder's organ in Bufo) because the progonads are not ovaries and sex is not really established. Indeed, even in the numerous cases where the progonad shows great predominance of male sex-differentiating influences (Fig. 11), and could legitimately be regarded as a testis, the developmental cycle remains unchanged, the sex cords appear, and the first-formed gonad gives place to a definitive testis.

SEX DETERMINATION AND SEX DIFFERENTIATION IN BIRDS AND MAMMALS

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In a brief review of this broad and intricate subject with its many points of debate, and even dispute, it will be necessary to some extent both to deal in generalities and to handle the subject in a somewhat arbitrary and at times in a dogmatic fashion.

Sex Determination

Briefly stated, our present knowledge leads us to believe that sex in birds and mammals is determined at fertilization. Some would associate sex determiners with chromosomes, others with differences in metabolic rate, but it seems possible, even probable, perhaps, that the two conceptions are really but different indicators of the same basic physiological condition and that we will find no contradiction when dealing with the two ideas.

Investigations of mammalian chromosomes have revealed in general a specific male and female chromosome constitution—an XX female condition, an XY male type, though some would deny the Y element in the male. In any scheme the male from a morphological aspect would be the heterozygous individual and genetic evidence bears out the supposition. The most modern chromosomal analyses of Painter ('22, '23, '24) on the opossum, monkey, man and horse would indicate such a condition to hold for mammals in general. Thus by segregation of the male XY elements and recombinations with the segregated XX female elements we have a morphological association of chromosomes with sex that indicates sex determination at fertilization or zygotic sex determination.

In the bird genetic evidence has for a long time led to the supposition that the female instead of the male is heterozygous for sex; theoretically the ova of birds are of two types, whereas all spermatozoa are alike. Within the last year or two the work of Stephens, Hance ('24) and of the Russian zoologist Shiwago ('24) demonstrates conclusively the ZZ male constitution and the WZ female type; thus genetic evidence for female heterozygosity is confirmed by morphological data. Riddle ('17) has given us a physiological demonstration of two classes of ova—a high storage, low metabolic type developing into females and a lower storage capacity, higher metabolic rate eggs producing males. Both the morphological and physiological evidence confirms the earlier genetic evidence and leaves little doubt that sex is determined at fertilization.

Granting, however, the zygotic determination of sex we are faced with graded expressions in differentiation (see Lillie '17b). Sex is coming to be recognized as having a quantitative as well as a qualitative aspect, and the expressions of latent possibilities of both sexes are often encountered in which elements of the two different sexes have developed simultaneously. Ordinarily, there is a normal balance of sex determiners, whatever their nature, that lead to a more or less pure expression of primary sex differentiation; but an occasional disturbance of such a balance often allows characters of the recessive sex to appear. We should bear in mind, therefore, the distinctness of sex determination and sex differentiation. Primary sex determination in birds and mammals is usually followed by the development of a typical male or female. The zygotic sex-determining factors alone, however, appear to be limited in their capacities as differentiators of sex and a second and supplementary factor, which may be termed the hormonal factor, is recognized as of great importance. This factor is commonly considered to be elaborated as a chemical substance or substances in the sex gland and distributed through the blood stream; but it is thoroughly appreciated that its elaboration, at least its expression, is dependent upon a balanced physico-chemical relationship to some extent influenced by other organs of internal secretion.

Sex Differentiation in Birds

Examples of the two influences, primary zygotic factors and hormonal factors, are beautifully illustrated in sex differentiation in the bird. Specifically, in the Brown Leghorn breed of the domestic fowl, the cock, aside from the characteristic head furnishings (comb and wattles), spurs and psychic tendencies, possesses an outstanding feather pattern not only in feather form but in its brilliant coloration. The female, on the other hand, differs in head furnishings, absence of spurs and both feather form and color. To determine the value of the hormonal influence in this marked sex differentiation it is necessary to eliminate it so far as possible by gonad removal. If this is done sufficiently early we have to some extent a true expression of the potentialities of the zygotic sex factors as sex differentiators. So far, however, gonad removal has not been accomplished until after hatching and it is difficult to know to what extent these may have been active during embryonic life.

In the Leghorn cock early, and to some extent later, removal of the testis produces a capon differing primarily from a normal cock in the absence of sexual instincts, and smaller, less vascular head furnishings. The typical cock feathering remains unchanged in form and color, unless perhaps the length and brilliancy of feathers are slightly enhanced; spur growth is unaffected. Spurs and feather pattern, long considered dependent upon testicular secretions, are seen to be due not to hormonal influences but to be an expression of the zygotic factors in differentiation. From these results, therefore, we may conclude that the supplemental or hormonal factor in male sex differentiation conditions the psychic nature, the head furnishings and perhaps to some extent body proportions.

We have been inclined to think that hen feathering was due to the lack of testicular secretions; but removal of the ovary has changed these suppositions. Following ovariotomy spurs begin to grow; growing feathers are changed in form from the simple hen type to the long feathers of the cock, and the sober color is replaced by the brilliancy of cock feathering; the head furnishings resemble those of the capon to a large extent. The end result of changes after ovariotomy and castration, in each case removing the hormonal factor in differentiation, is the production of two birds very similar in external form and behavior. This type has been characterized by Pezard ('24) as a "neutral form" and by Lipschutz ('19) as an "asexual form." These workers would, therefore, throw the entire burden of sex differentiation on the hormones, but it should be remembered that the very development of specific hormone-producing tissues is in itself an expression of primary sex differentiation. Primary sex differentiation is therefore dependent upon zygotic factors, whereas secondary sex characteristics are largely dependent upon hormones for their differentiation. It will be appreciated that, in the hen, the ovarian hormones supplement the zygotic determiners by stimulating the development of hen feathering, by suppressing spur growth, by regulating specifically the head furnishings and by stimulating the development of the psychic behavior.

It can be shown that the hormonal factor is more powerful than the zygotic factors in the differentiation of the outstanding secondary sex differences by implanting the gonads of one sex into the opposite sex. Thus we know from the work of Pezard, Sand and Caridroit ('24) and Zawadowsky ('22) that an ovary transplanted into a castrated cock will stimulate development of hen feathering with consequent elimination of the brilliant cock feathering. Experimental gynandromorphs have been produced by plucking feathers from one side of the male bird along with, or following, ovarian transplantation; new feather formation under the ovarian influences will be of the hen-feathered type, whereas the old, unplucked feathers are of the cock type; an ovarian graft in the castrated male will produce the female head furnishings. In some of these radically influenced differentiations it is almost impossible to distinguish a modified cock from the hen, except perhaps for size, despite the male determination. The hormonal influences have so modified differentiation that apparently (though not really) the opposite sex has been developed. In no sense, however, are we justified in stating that sex has thus been completely reversed.

The opposite condition, or a masculinizing effect, may also be realized. An ovariotomized hen with a testis transplant may be apparently converted into a male bird with the feather form and color of the cock; with typical cock headfurnishings, spurs and to a large extent psychic impulses those of the cock.

All these modifications relate to differentiation of secondary sex characters and it appears that the hormonal factor has no influence on the primary sex characters or gonads *once these are differentiated.* Thus Pezard and others have shown that a functional testis and ovary may coexist without detriment or influence to either. In the broadest sense, therefore, a true hermaphroditic condition has been produced experimentally.

It can be shown that the primary sex characters (gonads and ducts) are capable of being influenced in their differentiation by hormonal agents if these act during embryonic development. Minoura ('21) has demonstrated that a gonad graft made on the chorio-allantoic membrane of a developing chick produces gonad and duct relationships that deviate widely from the normal. Thus introduction of testicular hormones into a developing female produces a modification of the ovary and Mullerian ducts to the extent that a reversal towards the male development is indicated; similarly ovarian hormones introduced into a developing male appear capable of influencing development in the female direction. There is not yet direct evidence that complete sex reversal can be brought about solely through the hormonal influence.

Recent reports of Riddle ('24) and Crew ('23), however, prove that a primarily sex-determined female may, under the influence of pathological conditions, suffer a complete reversal and become a male individual with functional testes, due, as Riddle would believe, to a radically reversed metabolic condition.

That certain latent potentialities of the male sex exist in a determined female (the so-called bisexual potentialities) has been shown recently in another way by Zawadowsky ('22), Benoit ('23) and Domm ('24). Complete removal of the persistent left ovary of the bird at an early age is sometimes followed by an increased growth of the undeveloped and normally non-functional right gonad or tissue closely situated. In this development it may produce a testis-like organ and in the most extreme cases all testicular elements, including spermatozoa, are found. This unexpected and phenomenal result is correlated with a bird of typical cock feathering, head furnishings, spurs and to some extent a male psychic nature; on removal of the regenerated testis the bird reverts to the capon type.

One may conclude, therefore: (1) that normally, zygotic sex determiners are able to carry primary sex differentiation to the stage of the production of a specific hormone producing tissue; (2) that under pathological conditions a differentiated ovary may subsequently be replaced by a functional testis; (3) that under certain conditions both types of gonads may be present simultaneously in the same individual as in hermaphrodites; (4) that the normal female following early ovariotomy may develop a testis capable of producing spermatozoa; and (5) that hormones produced by the developed gonad enhance sex differentiation by stimulating development of the secondary sex characters; these latter, correlated with the gonad, may be of male, female or mixed character and being dependent upon the gonad present are likewise subject to reversals.

In the bird, therefore, we have recognized zygotic sex determination, the bisexual potentialities and the influence of a secondary hormonal factor in sex differentiation.

MAMMALS

In the mammal, if we can accept the zygotic determination of sex as conclusively demonstrated and turn to differentiation of sex, we have again the fundamental zygotic sex factors to balance against the hormonal ones, fully realizing that the hormonal ones are but an expression or more complete development of the zygotic sex determiners.

Our attention should again be directed to hormonal influences operating (1) during embryonic development, wherein the primary sex characters (gonads and ducts) are influenced, and (2) during postnatal differentiation, wherein we are restricted to the secondary sex characters. Normally the gonads and ducts develop accordingly as the individual is a determined male or female and any influence affecting the primary differentiation must act during embryonic life. Once structurally formed it appears that hormones are incapable of modifying the gonads despite the fact that some non-hormonal influences may affect them.

We are restricted to a single clearly analyzed case of hormone influence acting during development in the mammal—that of the free-martin studied by F. R. Lillie ('17a) and Tandler and Keller ('11). Due to peculiarities in membrane relations, calf twins become so connected through membrane anastomosis that the blood of the two individuals is mixed. Only in the case of differently sexed twins is there any sexual modification and here specifically the female member of the pair is influenced. The ovary of such an individual (called a free-martin) fails to develop as would be expected in a zygotic female and instead is modified decisively in the male direction. The gonad instead of being a typical ovary may be highly similar in structure to an undescended testis; uterine tubes are underdeveloped or absent, Wolffian ducts being preserved instead; seminal vesicles appear. Despite this extreme modification, however, there is no direct evidence that complete sex inversion is possible under the conditions stated. The modifying influence in this case is believed to be hormonal, that the hormone is produced in the testis of the male of the pair of twins and carried in the blood stream to the female member of the pair, thus influencing the development of the latter. It would thus appear that hormonal modification of a determined primary sex character is possible *during its formation,* but we are without substantial evidence that such is possible *after primary differentiation.*

Hermaphrodites in mammals (excluding the freemartin) are not known to be results of hormonal influences. Primarily the condition appears to be due to unbalanced zygotic factors with the appearance of both gonad types; subsequently, hormones from each may enhance the differentiation of secondary sex characters with the consequent appearance of maleness and femaleness in the same organism. Chromosomes have not been described in such cases nor are the metabolic conditions known, and the chief interest in such cases here is to emphasize the potentialities of each sex in the zygote; under normal conditions one set of potentialities predominates, the other being recessive. We do not know how this zygotic balance is so disturbed at times that both potentialities are expressed. Conceivably it may be based upon metabolic relations or again on other unknown conditions.

In postnatal differentiation in mammals objective results from experimentation have been less definite and striking than with the bird. This is due in large part to a lack of specific sex indicators in the ordinary laboratory animals on which the majority of investigations have been conducted. Steinach ('12, '13, '20) has employed body weight, body length, fat deposition, hair coat and the psychic nature, including sex inclinations, pugnacity, etc., in masculinized and feminized rats and guinea pigs as a criterion of sex. Under careful analyses body weight and length, as I have shown by specific data (Moore '19, '21, '22), are subject to such wide individual variations as to be wholly inadequate as sex indicators excepting possibly where comparisons are capable of being carried out on a large population of animals under specific conditions. Hair coat and fat deposition are entirely inadequate as qualitative expressions of sex. Psychical disposition is a variable entity subject to wide variation, and aside from the most general and outstanding sex inclinations are to be avoided as indicators of sexual conditions. Mammary glands are of considerable importance in registering changes in hormonal effects. Outstanding secondary sex characters are, therefore, largely restricted to internal structures, such as seminal vesicles, prostate, penis or its homologue the clitoris, vas deferens, uterine tubes, etc.

Through castration experiments of Bouin and Ancel ('04), Steinach ('12), Sand ('19), Lipschutz ('19), the writer and others we learn that the appearance and persistence of most secondary sex characters of the male are dependent upon the testis. General conditions can be illustrated by the non-development of horns in some castrated ruminants, the underdevelopment of the copulatory organs, prostate and seminal vesicles in practically any castrated male; body proportions in some forms are altered through castration; hair distribution, size of the larynx and other conditions may be altered in the human after castration. Many examples of similar nature could as well be given on the female side after ovariotomy. But the fact of such modifications after gonadectomy emphasizes also the fact that the supplementary or hormonal influences are the chief factors in the production of many of the most outstanding sex differences. Lipschutz would believe that all sex differentiation and even sex determination is dependent upon hormones, thus postulating a primitive asexual form (or soma) subject to primary differentiation in either direction. There are many facts, however, that would discourage such an extreme view of the potency of hormones.

Experimental modification of sex differentiation in the mammal by hormones have been attained almost entirely by sex gland transplantation or removal. Slight and somewhat questionable indications have followed injections of extracts or emulsions of gonads, but on the whole this line of experimentation has been peculiarly barren.

In complete ovariotomized females Steinach, Lipschutz, Sand and the writer have described varying degrees of masculinization by testis grafts. These degrees, however, are based upon the visible sex indicators and herein lies the difficulty of application. Apparently, each hormone is sex specific and acts only upon those structures which it normally affects. Thus the uterus and mammae are unaffected by testis hormones. The only morphological effect detectable under such conditions in these females is on the homologue of the copulatory organ or clitoris. These authors as well as the writer have described in the guinea pig an increase in the size of this structure to such an extent that it resembles the male organ more than the female.

The psychical transformation, many times described, I personally consider a very questionable indicator. The fighting instinct and the disposition of a rat or guinea pig are such variable reactions that they appear entirely worthless. The sexual instincts, if interpreted broadly, may offer a somewhat more adequate criterion. There is considerable positive evidence that sexual instincts of a zygotically determined female may be developed under hormonal influences to a decided male type even to the point of faithful reproduction of a complicated and distinctive copulatory pattern; these reactions are in my experience totally foreign either to normal or ovariotomized females and are to be considered as having been conditioned by the introduced hormonal factor.

The reverse condition also holds true within limits—*i.e.,* a castrated male may undergo differentiation in a female direction under the influence of ovarian hormones. Chief among somatic modifications is an abnormal growth of the mammary glands in the male guinea pig, to the point of milk secretion, according to Steinach and Sand.

This appears to be the only morphological character capable of an unquestionable modification. Ovarian secretions do not affect the penis, prostate or seminal vesicles which in the absence of the testis remain underdeveloped. Latent homologous characters are, therefore, stimulated, but heterologous characters are unaffected.

Sex instincts are more confusing in experimental feminization than in masculinization, due to the fact that often the male impulses are acquired despite early castration and may persist for long periods of time after late testis removal. I have observed castrated male guinea pigs with ovarian grafts so effective as to influence mammae development to the size of normal females, that still retained the male psychic tendencies sufficiently persistent that one would judge them to be normal males from reactions to other animals; and this with an entire lack of any demonstrable female psychic tendencies.

The bisexual potentialities, therefore, though to a large extent latent for one sex under normal conditions, are capable of development to a certain extent when the proper hormones are introduced experimentally. In hermaphrodites the primary differentiation often expresses the bisexual potentialities by development of ovatestes or ovary and testis in the same animal, due to unbalanced zygotic factors; specific hormones from each sex gland may then further intensify the mixed sexual condition by causing the appearance of both types of secondary sex characteristics.

Experimentally, some secondary sex character intermixtures can be produced by the simultaneous grafting of both sex glands into a castrated individual, or again by transplantation of a gonad into a normal animal of the opposite sex. Thus a male guinea pig may be produced having a normally functioning genital tract and well-developed mammae; or a female with functional reproductive system may carry a testis graft having little or no visible demonstration of effects on secondary sex characters, but the graft itself undergoing the processes of gamete production. Sand in particular believes he can recognize psychic bisexual expressions at the same time. Thus he reports a guinea pig acting at one moment as a male and immediately thereafter as a female. It is possible, however, to become overenthusiastic in such interpretations.

It should again be emphasized that, as in the bird, the primary sex characters appear capable of striking experimental modification during the course of their embryonic development by hormonal agencies; but after their primary differentiation we have no evidence that secretions from one gonad have any effect upon the other. I have shown that testes are perfectly normal when ovarian grafts have been present in an active condition in the male for months. Similarly, females will reproduce normally when carrying large testis grafts before, during and after pregnancy. Sand first showed that ovaries may be grafted into the substance of the testis and both glands remain in a normal condition; this has been substantiated both by myself and Lipschutz.

In review it may again be stated that to the best of our knowledge sex determination occurs in the zygote; and as Lillie ('23) has stated we may make the "assumption that the zygotic sex-determining factors are also sex-differentiating factors. . . . These factors are reinforced very early by hormone production." There is a great deal of direct and indirect evidence that the sex-determining, as well as the sex-differentiating factors have a quantitative as well as a qualitative aspect and that the zygote contains the potentialities of both sexes; this is beautifully illustrated in sex intergrades or hermaphroditic forms. The bisexual potentialities are clearly demonstrated by grafting the opposite sex gland into an individual of the bird and to a somewhat lesser extent in the mammal. Experimentally, therefore, we can control to some extent sex differentiation of a mammal or bird and it is not beyond the possibilities that further knowledge will provide us with instruments having much greater possibilities than we can at present anticipate.

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SHORTER ARTICLES AND DISCUSSION

THE EXISTENCE OF DOUBLE SPIRAL CHROMATIN BANDS AND A "BOUQUET" STAGE IN TRADESCANTIA PILOSA LEHM.

(Preliminary note)

THE difficulties which accompany the obtainment of adequate fixation of members of the genus *Tradescantia* for critical cell study, have been noted by several cytologists. During the past two years, the writer has experimented with a lengthy, graded series of fixing agents in an attempt to obtain good preparations of both somatic and meiotic cells of *Tradescantia pilosa* Lehm. In addition to the paraffin and celloidin methods, the smear method, recently described by Taylor,[1] was employed. These experiments reveal certain details of structure, which will be briefly mentioned here, prior to the publishing of a more detailed description.

In both somatic and meiotic phases, the chromatin is present in the form of a double spiral band. Indications of this structure have been observed in prophase, metaphase, anaphase and telophase chromosomes. It appears to be temporarily lost at the time of the division of the chromosomes to immediately reestablish itself in the daughter chromosomes.

The same spiral arrangement of chromatin has been observed in cells of *Rhoeo discolor* Hance.

There is no evidence in the heterotypic prophase of *Tradescantia* that synizesis is a natural occurrence. That, so often described aggregation of the chromatin material, frequently called synapsis by plant cytologists, does not appear in well fixed material. Instead, there appears a well defined "bouquet" stage, similar to that frequently figured and described by animal cytologists. A distinct attachment plate is visible at one side of the nucleus from which the spireme loops out into the nuclear cavity. It is important to note that in animal cells the "bouquet" stage associates itself with the presence of the centrosome.

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[1] Taylor, William Randolph, *Bot. Gaz.*, LXXVIII: 236–238. 1924.

A BALANCER IN LARVAE OF AMBLYSTOMA TIGRINUM

THE absence of the balancers has been regarded as a distinguishing characteristic of the larvae of *A. tigrinum.* Balancers are found in other larvae, *Amblystoma opacum, jeffersonianum* and *punctatum.* Careful study of developing embryos of *A tigrinum* has shown that rudimentary balancers exist in some individuals of this species for a very brief period.

The eggs from which these embryos developed were laid in an aquarium by individuals secured last year in the axolotl stage from collectors in Wisconsin. These animals metamorphosed after being received in this laboratory and upon molting this spring displayed the striping characteristic of *Amblystoma tigrinum.*

The development of the eggs was observed closely and at that stage of development at which the first reflexes appeared (corresponding to Harrison's Stage 37)[1] a slight outpouching was noted in the region just posterior to the eye. The growth of this outpouching was studied. From its form and position in the mandibular region a relationship to the balancer found in other forms was surmised. The subsequent development and morphology showed beyond question that this was the case.

The balancers are present only during a very short period of development and are generally lost to external observation during the growth from stage 37 to stage 38, a period of six to eight hours at normal room temperature. Further studies on the internal development of this structure are in progress.

Aside from the importance of balancers in the classification of species, there have been several considerations of a theoretical nature advanced as to their function and significance. It was suggested by Clark ('80),[2] that the balancer was developed in order to keep the head and gills raised from the mud on which the animal normally rests during development and therefore more readily accessible to the supply of clearer water; and further, that these structures caused the elevation of the pericardial region above the substrate, thereby eliminating interference with the heart beat. Latta ('19)[3] seems to suggest that either of these explanations is plausible from his own observations.

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[1] *Jour. Exp. Zool.,* Vol. 25.

[2] **Studies from the Biological Laboratory of Johns Hopkins University, No. 11.**

These theoretical deductions concerning the significance and function of the balancer are not supported by the observations on *A. tigrinum*. There is no reason to suppose that larvae of this species do not require protection from the same adverse conditions as found in other species, yet the balancers are exceedingly rudimentary, non-functional and transitory.

Experimental studies in which the balancer bud, as the process extending behind the eye may be correctly termed, has been stimulated either by dividing the bud itself with a fine pair of scissors or by pricking the regions slightly anterior or slightly posterior to the bud region have given rather interesting results. The division of the balancer bud without the implantation of any inert material will not bring about the formation of a double balancer. The resorption of these structures, however, can be delayed by division of the balancer bud, and the period of their existence can be prolonged 24 to 36 hours. Stimulation of the region anterior to the bud also prolongs its existence. Stimulation of the materials posterior to the bud gave no results.

In the larvae of *A. punctatum* the balancer is broken off at the 14 mm stage. The proximal portion or base is later slowly resorbed. The transitory balancers of *A. tigrinum* are lost by resorption.

It must be concluded that at least in certain races of *Amblystoma tigrinum* a transitory balancer is found. This structure is never of functional importance in the form here studied. It is possible that other gradations in its development may be found in other races. The presence or absence of balancers is, therefore, not necessarily diagnostic of any one species of Amblystoma.

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[3] *Anatomical Record,* Vol. 17.

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SYMPOSIUM ON SPECIES AND CHROMOSOMES[1]

SPECIES AND CHROMOSOMES

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IT is only since the beginning of the present century that the progress of biology has rendered possible the discussion of this topic. When the "Origin of Species" was written, chromosomes were unknown, and the study of nuclear division and the relation of chromosomes to the life cycle only began in the seventies and eighties of the last century. The names of Flemming, Strasburger, Boveri and many others are connected with the early work on chromosomes, when alcoholic fixation and methods of sectioning first made possible their study.

In the last fifteen years particularly, the alliance between cytology and genetics in the experimental study of heredity and variation has become increasingly intimate. Beginning with the recognition of chromosome segregation in meiosis as the basis of Mendel's law of heredity, this was soon followed by the discovery (1906) of definite relations between chromosome numbers and mutations in Oenothera. Since 1910 Morgan and his colleagues have built up a combined system of genetical and cytological results in Drosophila which is unequalled in any other organism. Their theory of linkage and crossing-over has brought the phenomena of meiosis, chromosome structure and heredity into still closer relationship More recently Blakeslee and Belling have discovered chromosome relationships in Datura which parallel many of those formerly obtained with Oenothera, and in a striking series of experiments have extended those results in various directions.

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[1] At the request of the Sectional Committee having the discussion in charge, Professor E. C. Jeffrey has secured from their authors most of the contributions to the discussion on "Species and Chromosomes," which were given at the Toronto meeting of the British Association for the Advancement of Science. There has been some delay on account of the illness of two of the most important contributors and one of them is still too infirm to have supplied as yet a summary of his remarks. It will be noted by the reader who is familiar with the names appearing in connection with the symposium that Mr. Julian Huxley and Dr. Heslop Harrison, two zoological contributors, are not represented. Mr. Huxley was absent from the discussion and indisposition has prevented Dr. Harrison's paper arriving in time for inclusion in the symposium.

In recent years, a number of predictions of peculiar cytological conditions have been made on the basis of genetic experiments, and afterwards confirmed by observation of the cells. An example is the work of Bridges with strains of Drosophila having an extra chromosome. This condition arises, as was first shown in Oenothera, through failure of one pair of chromosomes to enter separate nuclei in reduction, a process which is now known as non-disjunction.

The following may now be regarded as generally accepted facts concerning the chromosomes. They are bodies of relatively fixed size, shape and point of attachment to the spindle fibers. Homologous pairs usually separate in meiosis and thus furnish the basis for simple Mendelian behavior. Their number is generally fixed for each species so far as the germ-lineage is concerned. The relations of genetical crossing-over to chromatin behavior are complicated and need not concern us here Aberrant chromosome numbers arise in various ways and produce forms with different external characters Such forms have been specially investigated in Oenothera, Drosophila and Datura. The most striking changes found are tetraploidy ($4n$ chromosomes), triploidy ($3n$) and non-disjunction leading to forms with an extra chromosome ($2n+1$, $4n+1$, etc.). These conditions occur in all three genera, except that tetraploid forms have not been observed in Drosophila. The condition found in Oenothera particularly furnished the first experimental basis for a comparison of the chromosome numbers in various wild and cultivated species of plants.

When we examine the chromosomes of related species of plants and animals, differences in number or in size and shape of the chromosomes are often found. How have these differences originated? The answer to this question has already given us the beginnings of a chromosome phylogeny, which can now be soundly based on analogy with experimental results. The case of *Oenothera gigas* furnished the first experimental clue to the origin of tetraploidy. Many wild tetraploid species of plants are now known, and they must have arisen in some such way as *Oe. gigas* appeared, *i.e.*, as mutations. The tetraploid and higher polyploid species are turning out to be surprisingly common in plant genera, though apparently much less frequent in animals.

Let us attempt to classify the various types of change which are indicated to have taken place in the phylogeny of chromosomes by the comparison of related species or genera. We may mention:

(1) Polyploidy, or the development of higher multiples of chromosome numbers.

(2) Transverse segmentation or fragmentation of all the chromosomes, or of particular pairs. A phenomenon of this nature seems to have occurred on a large scale in the phylogeny of the Liliaceae.

(3) The end-to-end fusion of certain chromosomes. This is particularly clear, both from genetical and cytological evidence in certain species of Drosophila, and appears to have happened in various other cases.

(4) Irregular division or non-disjunction may lead to forms with additional pairs of chromosomes.

(5) The recent genetic evidence from Drosophila and the behavior of the chromatin in such forms as *Oenothera lata* indicate that not only may chromatin fragments enter a nucleus and afterwards divide, but also that portions from one chromosome may become attached to an-

other and thus alter the genetic relationships of the factors concerned. This process is one which must also be considered as important in the evolution of species.

(6) In the genus Carex, Heilborn (1924) has shown that the haploid numbers in forty-four species run from 9 almost continuously to 42 and 56, but without multiple numbers. Heilborn suggests that one of the most important methods of chromosome increase in Carex has been by mutations of the *Oenothera lata* type followed by a division of the extra chromosome.

(7) Certain species and even genera with new chromosome content have probably arisen through crossing. Cultivated maize may have arisen from a cross between the Mexican Euchlaena and some species of grass belonging to the Andropogoneae. J. Clausen finds that *Viola tricolor* and *V. arvensis* have, respectively, 13 and 17 chromosomes as haploid numbers. In a cross the F_1 plants have 30 chromosomes, but in certain of the pollen mother cells 47 were found, *i.e.*, $13 + 17 + 17$, a condition of semi-tetraploidy.

It is thus becoming clear that chromosome changes have arisen in a variety of ways, including transverse segmentation or fragmentation, end-to-end union of certain pairs, gradual diminution and disappearance of certain chromosomes, non-disjunction, rearrangement of portions of chromosomes, crossing of species having different numbers and polyploidy.

From the work of the last fifteen years, it has become clear that polyploidy has played an important part in the evolution of many plant genera and families, and that it is of particularly common occurrence among the varieties of cultivated plants. If tetraploidy can be brought about, as seems likely, by a lowering of temperature when the fertilized egg is beginning to divide, then one may perhaps understand its prevalence in plants and its relative infrequency in animals where temperatures are more controlled. But that it can be produced in other ways is shown by its experimental production in mosses by wounding (Marchals), in Solanum by grafting (Winkler), and semi-tetraploidy at least by crossing.

The fact that such notoriously polymorphic genera as Rosa, Rubus and Crataegus are all found to show polyploidy in a high degree is a discovery too fresh for its significance to be fully realized.

Similarly in the evolution of various other genera and families, higher and higher polyploid numbers have been reached. Thus in the genera Chrysanthemum, Rumex and Rosa, species are found which are $2n$, $4n$, $6n$ and $8n$ in their chromosome constitution. Some of these genera also have $3n$, $5n$ and even $10n$ species. Many of these species are apogamous, and where the chromosome number is an unbalanced one, as in $3n$ and $5n$ species, it can only be maintained by apogamous or vegetative reproduction. Thus these unbalanced species must always have been apogamous from their origin.

The conditions in the wheats, where $n = 7$, may be briefly mentioned, Einkorn wheat (*Triticum monococcum*) is diploid, the species of hard wheat are tetraploid, and the Vulgare wheats (soft), which comprise our highest yielding modern sorts, are hexaploid. When members of different groups are intercrossed they produce sterile hybrids. All three groups go back to prehistoric times. The tetraploid wheats presumably arose, like *Oenothera gigas,* through a sudden doubling in the number of chromosomes. The most probable hypothesis of the origin of the $6n$ condition is that it came from a $3n$ hybrid between diploid and tetraploid species, one such hybrid having doubled its chromosomes, perhaps in the fertilized egg, and so restored its fertility. In certain cultivated varieties of mulberry, the $3n$ condition is maintained by apogamy, and the same is true of the wild species *Eupatorium glandulosum* and *Erigeron annus.* Similar polyploid conditions are being found in many cultivated plants, including bananas, pineapples, oats and dahlias, so that much more evidence concerning the precise manner of origin of these conditions ought to be obtainable.[2] Enough is already known to show that several different kinds of, for example, tetraploidy exist.

The development of sterility between species has undoubtedly played an important part in evolution. How this condition has come about is still a matter of speculation, but we are beginning to get some light on the subject. The old view that forms which produced fertile hybrids must belong to the same species has completely broken down, as has the converse conception that in plants sterile pollen is a proof of hybridity. One of the most interesting features of tetraploidy is its relation to the development of sterility. As has been pointed out elsewhere,[3] if a tetraploid form such as *Oenothera gigas* arises in nature, its hybrids with its diploid parent must contain an unbalanced chromosome content, and this leads to the dropping out of chromosomes in later generations until the diploid number is restored.[4] The triploid form can only perpetuate itself by developing apogamy (as certain triploid species and varieties have done) or by doubling its chromosomes, thus producing a hexaploid form. In all three cases new centers of stability will be reached, which are starting points for fresh variations. The essential conditions for physiological isolation are produced.

In this way tetraploidy and the higher forms of polyploidy, as well as other types of chromosome change, have been of great importance in evolution, since they have the effect of physiological isolation even when there is no isolation in space.

The study of chromosomes thus leads to results of far-reaching importance for evolution. It is of the highest importance for taxonomy in determining the relationships of species and genera. With advancing knowledge and quicker methods of examining chromosomes, the time should come when the description of a species is not considered complete until the morphology of its chromosome group is known, for visible chromosome differences, when they occur, throw important light on relationships. With further knowledge we shall doubtless learn much concerning the phylogenetic nature of the various kinds of chromosome change which have been outlined here. They suffice to indicate that there is a fundamental evolution of the germplasm, of which changes in specific characters are merely the external expression. Already as regards tetraploidy these changes have been experimentally analyzed and we know something of the conditions which bring them about.

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[2] Some of these subjects have been more fully discussed elsewhere. Polyploidy, *Brit. Journ. Exptl. Biol.*, I, 153–182, 1924.

[3] *Nature*, 1922, vol. 110, pp. 179, 447.

[4] See Gates, "The chromosomes of a triploid Oenothera hybrid," *Ann. Bot.*, 37, 565–569, 1923.

Modern genetics has led us to deal, as Bateson has said, with the world of gametes which form the background for the visible inherited variations of species. The next decade of comparative and experimental work with chromosomes will bring the realization that in the secular changes of the chromosomes, however controlled or determined, we have a means of understanding how the cell unit, and through it the organism, has varied in structure in the past from species to species. This gives a new line of approach, of fundamental importance, in the analysis of the processes of evolution. It is to be hoped that its significance will be recognized, not only by experimental evolutionists as an additional line of evidence in tracing phylogenies, but also by taxonomists as a necessary element in their discrimination of genera and species.

From a phylogenetic point of view, two important principles have emerged from the work of recent years in experimental evolution and other fields. One of these is the principle of parallel mutations, which introduces a new element of fundamental importance into the reconstruction of phylogenies. The original case of parallel mutations experimentally determined was that of the *lata* mutations from *Oenothera Lamarckiana* and from

Oe. biennis, both mutants having 15 chromosomes and the same peculiarities of leaf and habit and sterile pollen, but each having the flower-size of the species from which it was derived. Innumerable cases of parallel variations are now known in plants, and many in animals. They appear to have originated as mutations. When their significance is appreciated by systematists they will have a profound effect on present conceptions of relationships and phylogeny, for they show that many similar variations have occurred over and over again in unrelated groups.

The other principle has been emphasized particularly by paleontologists in recent years.[5] We may call it *orthophylesis* to avoid the special connotations of previous terms. It consists in the recognition of longer parallel phylogenetic trends in different lines of descent. What relation, if any, these orthophyletic trends bear to parallel mutations remains to be investigated. But it is possible that some of them, at any rate, as pointed out earlier in this paper, may be the result of changes initiated in the chromosomes. In any case the comparative study of chromosomes offers one of the most promising lines of advance in experimental evolution.

CHROMOSOMES AND CLASSIFICATION IN THE GENUS *ROSA*

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Roses provide such a tantalizing array of forms to the taxonomist that it seemed a promising group on which to test the possibility of throwing light on classification by means of a study of cytological conditions. The results have amply rewarded the effort; for the work of Täckholm (4, 5) and of Heslop Harrison and myself (2) has shown that this line of research is capable of yielding very useful data both from a taxonomic and a general evolutionary aspect.

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[5] See Watson, D. M. S., Croonian Lecture, Royal Society, 1924 (*Nature*, June 7, p. 841).

In *Rosa* the fundamental chromosome number is seven and the majority of American forms have this as the haploid figure, being therefore diploid; among these are *R. carolina, R. nitida* and *R. setigera.* In the British Isles the only native diploid form is *R. arvensis.* Again in England we have only one tetraploid form, *R. pimpinellifolia,* whereas in America there are *R. blanda, R. lucida, R. pratincola* and other forms. The remaining American forms have twenty-one as their haploid number and are hexaploid. Thus the western species provide among themselves a good polyploid series with diploid, tetraploid and hexaploid forms. Such series have been described in other genera, but the greatest cytological interest is focussed on the eastern group, Caninae, to which most of our English species belong. This section is one of the greatest puzzles to the taxonomist, since the innumerable forms run into one another to such an extent that it is well-nigh impossible to define any species limits at all. For this reason we examined the forms cytologically as well as in the field to try to throw further light on the subject. The following is a brief account of the results obtained which are in some respects unique with regard to cytological behavior.

To illustrate this we may take for example a form from the sub-section Villosae, *R. mollis* will serve: Its somatic chromosome number is twenty-eight, exactly as in *R. blanda* or *R. pimpinellifolia* and it is therefore tetraploid. In the reduction division in the anthers the early stages show nothing to arouse suspicion that there is anything abnormal, but at the metaphase the chromosomes are very slow to arrange themselves on the equatorial plate and, when a particularly tidy plate enables the chromosomes to be counted, it is seen that, instead of fourteen, as might have been expected, there are twenty-one. To cut a long story short it was found that the central seven of these, which are larger, are bivalents, and the peripheral fourteen univalents. The bivalents promptly separate and pass neatly to the poles, but the univalents divide and follow them more slowly; and so, when the daughter nuclei are formed, many of the chromosomes are left out. These later form micronuclei. The same story is repeated in the next division, which is even more irregular. Finally up to eight pollen grains may be formed instead of a normal tetrad and most of these are abortive; only those which contain the seven chromosomes descended from the bivalents appear to survive and probably those with exactly seven are the most fertile.

The other subsections of the Caninae examined showed similar behavior except that the somatic count is thirty-five. Such a pentaploid species shows itself to be abnormal even in its somatic cells, since the chromosome count is not, as usual, divisible by two. The reduction division in the anther again shows seven pairs of chromosomes and this time twenty-one univalents.

In addition Täckholm (5) has described one or two scattered varieties in which the somatic figure is forty-two and there are again seven bivalents with twenty-eight univalents.

Considering the features shown by these forms—the only partial reduction in the heterotype division, the formation of micronuclei and later of extra pollen grains and the generally bad state of the pollen—we are irresistibly drawn to a comparison with known hybrids with parents of unequal chromosome numbers such as appeared in Rosenberg's classical work on *Drosera anglica* (3). Such hybrids are usually unfertile, though there are exceptions to this. Why then do the Caninae set good seed and persist as such a dominant race? The answer to this, I think, that they have adapted themselves to their peculiar condition in two ways.

(a) Nearly all the forms referred to, if not quite, can reproduce themselves apomictically, that is to say, they produce purely matroclinous plants by some method independent of fertilization and so perpetuate the race.

(b) Megaspore development is quite different from microspore development, as Täckholm has shown, in that the fertile megaspore normally retains all the univalents and seven units from the bivalents, so that, should the plant be fertilized by one of the seven chromosomed pollen grains, the fertilized egg would contain exactly the chromosome complement of the parent. Our investigation of the hybrid *R. coriifolia* × *R. lutetiana* has shown that this actually takes place at times, extraordinary as it may seem.

Additional support to the hypothesis that these ordinary members of the British Flora really are hybrid in origin may be obtained by examining the cytology of some of the known rose hybrids. In those with both parents diploid, such as *R. cinnamomea* × *R. rugosa,* or both parents tetraploid, as in *R. pendulina* × *R. pimpinellifolia,* the behavior is regular at the reduction division and the plants are fertile, but cross *R. cinnamomea* with *R. pendulina* and you obtain a triploid individual which, at the reduction division, gives seven bivalents and seven univalents in just the same way as in the Caninae forms described above. Again *R. pendulina* × *R. nutkana* has 14 + 21 chromosomes, of which fourteen pair at the reduction division in a similar way.

We have thus in the roses a group of plants with a base number of seven chromosomes in which there are several polyploid series: the first series comprises normally reducing forms with their somatic chromosomes in multiples of fourteen giving thus diploid, tetraploid, hexaploid and even, in one type of *R. acicularis,* octoploid forms.

The remainder of the roses have partial reduction only and fall into two groups according to the number of bivalents at the reduction division. The one series with seven bivalents contains forms which are triploid, tetraploid, pentaploid and hexaploid, and the second, with fourteen bivalents, contains pentaploids and hexaploids.

We have now to consider the question of the possible origin of these different chromosome complements, which is, in this case at any rate, practically the same question as the origin of the species.

The Caninae section has been shown to be probably derived by hybridization at a relatively recent date but, for the origin of its various forms, it is necessary to assume an original polyploid series with forms up to the octoploid.

It needs to be considered if we have any evidence as to a possible method by which the original polyploid might have arisen from diploid ancestors. I shall proceed to show that some evidence is now available (2).

If you consider the possible crosses between a member of the section Caninae and a rose of some other section, since the pollen and embryo-sac of the former have a different complement of chromosomes it will be obvious that reciprocal crosses might be expected to have a different chromosome number.

Consider the hybrid *R. tomentosa* × *R. pimpinellifolia,* which is a not uncommon combination in the British Isles: if *R. tomentosa* is the female parent the chromosome complement should be $28 + 14 = 42$ and the plant will be strongly matroclinous. Such a hybrid we know in *R. Sabini,* which behaves exactly as might have been expected in its reduction division, resembling *R. tomentosa* except for the addition of seven chromosomes giving fourteen bivalents. This form is quite sterile, the flowers drop off at an early stage.

A search for a reciprocal hybrid among naturally occurring plants revealed *R. Wilsoni* growing at Bangor, N. Wales. Miss A. J. Davey, M.Sc., very kindly prepared for us slides of this form and we were thus able to examine its cytology. We expected to find $14 + 7 = 21$ chromosomes, since we have assumed that pollen grains with seven chromosomes are most likely to be fertile. To our great surprise we found that it possesses 42 somatic chromosomes and moreover shows almost no evidence of hybrid nature in its cytology. Its reduction division is almost like that of such a normal hexaploid form as *R. nutkana,* and moreover it sets good seed. Now had the parents possessed an equal number of chromosomes this might not have been surprising; as it is, some explanation is required. Carefully considering all possible hypotheses, we were drawn to the conclusion that the chromosome number had doubled, probably by a suspended division in the egg cell, and thereby given each chromosome a synaptic mate. The fertility is possibly dependent on this last factor.

If this hybrid has become fertile by doubling its chromosomes it suggests a possible origin for all the known polyploid forms in the genus; the balanced ones arising by hybridity and doubling, and later the unbalanced ones by hybridity followed by apomyxis.

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CHROMOSOMES AND SPECIES

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THE problem of the systematic value of the chromosomes, their number and shape, is a rather new one and it is hardly possible to make any general statements. But as Tischler and others have shown, a study of the chromosomes in smaller systematic groups may already be used for the elucidation of relationships.

From a cytological point of view pure species are characterized by a certain balanced diploid number of chromosomes. An analysis of the mode of formation of such balanced numbers will surely give some explanation of the importance of the relation: chromosomes and species.

In many cases new species may arise by new combination of genes, and one might then be justified in concluding that sometimes, by adding or combining new chromosomes to another balanced number, a new constant type would result.

Hybridizing may give new constant chromosome-combinations as a result. However, in the case of the *Drosera*-hybrid, a cross between two gametes, differing in number of chromosomes, the result was an offspring which in the heterotypic division gave nuclei with a very different number of chromosomes. In *Papaver* we have a very interesting case where crossing resulted in an increase in the balanced number of chromosomes. Miss Ljungdahl crossed two species with 7 and 35 chromosomes, respectively, in the gametes. The hybrid had 42 as diploid number, and 21 gemini were formed in the heterotypic division; back-cross to the one parent with 7 chromosomes gave an offspring with 28 chromosomes in the somatic division, and 14 gemini in the heterotypic division. Thus, the crossing-experiments gave as result types with a new balanced number of chromosomes; we may say from a cytological point of view, new species. The constancy in chromosome number is caused by allosyndesis, *i.e.*, conjugation between homologous parent-chromosomes and autosyndesis, which means conjugation between sets of chromosomes belonging to the same parent. Accordingly, we see that the pairing of chromosomes is determined by the degree of homology as well as by the descent from sperm and egg.

In the case of non-disjunction in the heterotypic division the two non-conjugating chromosomes may behave in different manners:

(1) They may both go to the same pole, and, as regards the number of chromosomes, the result will be that

after the homotypic division the number of chromosomes will be higher in two tetrad-cells and smaller in the other two; (2) if the non-conjugating or single chromosomes in the metaphase are situated in the equator they will often split longitudinally in the anaphase and consequently the result will be an increase in number of chromosomes in the 4 tetrad-cells; (3) sometimes these single longitudinally split chromosomes are situated nearer one pole in the anaphase, and will be included in the daughter nucleus at interkinesis. In this case extra chromosomes are added to one of the daughter nuclei.

These irregularities refer to the first or heterotypic division. In the second, or homotypic one, the chromosomes split longitudinally, and the same irregularities as regards the single chromosomes often occur. The single chromosomes which split longitudinally in the first division will often split again in the second one, but a little later than the gemini chromosomes, thus causing delayed chromosomes. Accordingly, the number of chromosomes in the gametes must vary. In one *Hieracium* species with the somatic number 36 I counted from 15 to 25 chromosomes in the pollen-cells after reduction division with non-disjunction. One should have expected only 18.

After fertilization the offspring will form individuals with a very different number of chromosomes. In most cases perhaps the number of gemini in the heterotypic division will be the same as in the parent-individuals, with or without single chromosomes. However, in other cases the fertilizing gametes may have some single chromosomes homologous. The result will be that in the heterotypic division the homologous singles pair to gemini, and the number of gemini, *i.e.*, the balanced chromosome-number, will increase.

In some crossing-experiments with species of the genus *Hieracium* I have found such an increase in number of gemini realized. The female plant was itself a hybrid with 42 chromosomes, 36 of which paired to 18 gemini—

in the following called gemini-chromosomes—and 6 single ones in the heterotypic division. The male-parent had typically 18 gemini and some singles. Nevertheless, in the pollen-cells I found the chromosome-number varying from 15 to 25. In the F_1-offspring in some individuals I counted 25 gemini and some singles—this increase probably being due to a pairing of homologous single chromosomes in the gametes. In one hybrid between two *Hieracium*-species, which normally have 18 gemini, but also non-disjunction, I counted 33 chromosomes, and in the heterotypical division 15 gemini and 3 unpaired chromosomes. Probably the pollen- and egg-cells of the parent-species had 18 to 15 chromosomes. Hence, by non-disjunction we have obtained a type with a lower balanced number than the parent plants, thus forming a cytological basis for a new species.

A very interesting problem has turned up lately in consequence of recent years' very increased knowledge on the number of chromosomes in related species and genera: Is there any relation between the number of chromosomes and the phylogeny of the species?

One has recently tried to support the theory that in small groups species with the higher chromosome-number should represent the younger types; phylogenetical lines should go from groups with low number to those with higher ones. According to my opinion it is very difficult to make any decision yet. I am inclined to think that in small systematic groups higher numbers are derived from the lower ones. But this does not mean that a species with a small number must therefore be considered as a more primitive species than another one from the same group, but with a higher number. And in fact, it is not yet quite clear if not a higher chromosome number in some cases could be acquired by transverse section of chromosomes; if so, one might also expect that chromosomes could fuse end to end, which would result in a decrease in chromosome number.

POLYPLOIDY AND THE ORIGIN OF SPECIES

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It is known as a result of the investigations of recent years that frequently different species of the same genus may manifest diverse equipments of chromosomes in their cell divisions. For example, the species of Chrysanthemum, according to Japanese investigations, may exhibit 9, 18, 27, 36 and 45 in their reproductive mitoses and their sporophytic cell-divisions which represent the double of the gametic numbers are as a consequence known as diploid, tetraploid, hexaploid, octoploid and pentaploid. In addition to this type of polyploidy there is another, well exemplified by large genera of the Rosaceae. In the Rosaceae, as a result of the very interesting investigations of Rosenberg and his student, Täckholm, in Sweden, of Blackburn and Harrison in England, and of Longley in America, we know of the existence not only of diploid, tetraploid, hexaploid and octoploid species but also of species which are triploid and pentaploid. In the gametophytic mitoses of the triploid and pentaploid species complications are introduced by the fact that the sporophytic number of chromosomes when divided by two does not result in an even multiple of the gametophytic number. Since it is apparently desirable to have names to describe these two types of polyploidy for the even multiples, as in Rosa and other genera, the term artioploid may be suggested, and for the uneven multiples the corresponding vocable perissoploid.

Before passing on to the consideration of the relation of polyploidy, artioploidy and perissoploidy to the origin of species, it will be well to refer to still another type of abnormality in chromosome equipment, which is presented by plants. A notable illustration of this condition is furnished by the very recently published investigations of Heilborn on the huge sedge genus Carex. Here the numbers of chromosomes in mitosis varies greatly and does not come under the law of multiples. The numbers, for example, run 9, 15, 16, 19, 24, 25, 26, 27, 28, 29, 31, 32, 33, 34, 35, 36, 37, 38, 40, 41, 42 and 56.

A similar state of affairs is found in the compositaceous genera Lactuca and Crepis as well as in other instances, which need not be mentioned here, since we are merely exemplifying general conditions. The non-multiploid variation in number of the nuclear chromosomes may advantageously be termed dysploidy, since it represents variations in number which are irregular and do not conform to any definite numerical principle. Dysploidy is a phenomenon in general much less common than artioploid or perissoploid polyploidy.

In contrast to artioploidy, perissoploidy and dysploidy as defined above, we have many species which agree absolutely in their chromosome equipment; this for example is true of the genera Pinus and Lilium, which in general have the same number of chromosomes in their various species. In the genus Oenothera the number of chromosomes is usually seven (gametophytic) but variations from this condition are known in the case of certain of the so-called mutants or elementary species, where the chromosome equipment may be artioploid, perissoploid or dysploid in accordance with the terminology used above. It is the outstanding service of DeVries to have called the attention of biologists to these so-called mutants. The explanation of their origin is as yet unsettled, but resolution of this problem seems now measurably in sight. It will be clear, I hope, from what has already been said that in genera and species which are somewhat uniform in their chromosome equipment, variations from the normal may sometimes be found, which come conveniently under the headings perissoploid, artioploid or dysploid or even all these. These departures from the typical condition may conveniently be united under the general term polyploidy, although that vocable must then be used in a somewhat broader sense than is usual. Using polyploidy in the sense above defined we have definitely reached the problem which falls to my share in the present symposium. There seems to be no doubt that polyploidy in the broader sense defined above is in certain cases definitely correlated to the phenomenon of the appearance of new species. The problem before us is to attempt to explain the origin of polyploidy in terms of known facts and thus the origin of certain kinds of species, not only of the so-called mutants or elementary species, but also of many of those species commonly recognized by the systematist.

It has been a common practice during the past two decades to cover our ignorance of the origin of polyploidy and associated phenomena by the use of the term mutation. The huge experimental activities in the case of Oenothera and Drosophila have to my mind been much less fruitful for our science than they would otherwise have been, on account of the often too limited point of view of mutationists. A great soldier more than a hundred years ago was wont to remark that God was on the side of the big battalions. Without wishing to endorse entirely that unmoral sentiment, I believe that it is beyond question that the study of large genera along both comparative and experimental lines is more likely to throw real light on certain modes of origin of species than has the intensive and extensive investigation of any of the so-called mutating species of Oenothera, Drosophila and Datura.

Since the general subject before us this morning is large and my time is limited, I shall focus attention on the Rosaceae as represented by the genera Rosa, Rubus and Crataegus. These are all large and polymorphic genera and exemplify in high degree the phenomenon of so-called mutation. It is clear from the study of experimentally produced hybrids that all the types of polyploidy may result from hybridization, namely, perissiploidy, artioploidy and dysploidy. Since the species of the Rosaceae often present the morphological characteristics of hybrids, it is reasonable to assume that to a very large extent the origin of species, in this large order, has taken place as the result of the crossing of formerly existing species. This hypothesis gains strong support from the surprising ease with which hybrids are produced among the Rosaceae, both in nature and under experimental conditions. For these obvious and recent crosses, I have earlier suggested that the use of the term phenhybrid and for those older hybrids, to which the systematist dignifies with specific names, I have proposed the appellation crypthybrid. The work carried on in Professor Rosenberg's laboratory and in my own on the Rosaceae furnishes ample evidence of the hybrid origin of many recognized species. Recently the cytological investigations of Dr. A. E. Longley, carried out on the rich material growing in the Arnold Arboretum of Harvard University, make it clear that many species of Rubus and Crataegus are either phenhybrids or crypthybrids. There is, however, one direction in which question of the validity of these conclusions is likely to arise. Frequently on occasions such as the present geneticists have expressed themselves in an uncomplimentary manner regarding the value of morphology in evolutionary investigations. Although it is often evident that criticisms of this sort reveal some narrowness of outlook and even superficiality on the part of their authors, it will be well in this connection not to base our conclusions on morphological evidence alone, although this is by far the most utilizable and widely serviceable, in reaching general results regarding the origin of species, preeminently a problem for the morphologist.

The genus Rubus seems to be of very special importance at the present time, from the standpoint of the study of the origin of species in the Rosaceae. Professor Hoar, after an extended study of the organization of the pollen in the various American species belonging to the genus, particularly the Eubatus or Blackberry section, came to the conclusion that the species of Rubus were very largely of heterozygous or hybrid origin.

Some years later Brainerd and Peitersen, as the result of the systematic study of the blackberries of New England, came to the conclusion that there are many natural hybrids between recognized species of Rubus—these not infrequently in cultures, segregated back to their suspected parental forms. These hybrid or phenhybrid species are illustrated by *Rubus andrewsianus, R. orarius, R. frondisentis, R. glandicaulis, R. flavianus, R. fraternus, R. abbrevians, R. jeckylanus, R. vermontanus, R. viridifolius, R. junceus, R. groutianus, R. arenicola, R. alleghaniensis, R. argutus, R. pergratus, R. frondosus, spinus,* and *R. plicatifolius.* These authors recognize as more or less good species of Rubus the following: *R. alleghaniensis, R. argutus, R. pregratus, R. frondosus, R. canadensis, R. elegantulus, R. vermontanus, R. setosus, R. hispidus, R. flagellaris, R. baileyanus* and *R. enselenii.* Even in the last group, however, the pollen is often up to ninety per cent. sterile and the seedlings frequently show a considerable degree of variability, although in general true to the specific type. Apparently on systematic grounds some even of the second group must accordingly be regarded as crypthybrids.

In a second article appearing under the name of the junior of the two authors cited in the preceding paragraph, the pollen conditions in a number of Rubi are described. A greater or less degree of sterility marks all except *R. alleghaniensis.* The examination of the pollen was supplemented by germination experiments in which the results corresponded very closely with those derived from the morphological study of the pollen. In this and the preceding article the authors note the frequent Mendelian segregation of the seedlings of suspected hybrid forms towards their parents. For example, *R. andrewsianus* segregates to *R. alleghaniensis* and *R. argutus; R. arenicola* to *R. baileyanus* and *R. frondosus; R. cubitans* to *R. hispidus* and *R. vermontanus; R. flavianus* to *R. alleghaniensis* and *R. elegantulus; R. groutianus* to *R. elegantulus* and *R. setosus; R. jacens* to *R. hispidus* and

R. setosus; R. junceus to *R. canadensis* and *R. setosus; R. orarius* to *R. alleghaniensis* and *R. canadensis; R. permixtus* to *R. alleghaniensis* and *R. hispidus* and *R. trifrons* to *R. hispidus* and *R. setosus.*

The cytological investigations of Longley on the genus Rubus show a very interesting agreement with the general systematic and genetical results obtained by Brainerd and Peitersen. For example, the specific constancy of *R. alleghaniensis* from the systematic standpoint and its good pollen have a striking parallel in its cytological behavior, for in this species the gametophytic chromosomes are seven and there are no abnormalities in the heterotypic and homotypic mitoses, such as are often found in known hybrids. *R. argutus Link* comes within the same category, for a high degree of fertility, as exemplified by good pollen, has its counterpart in a diploid condition of the sporophyte and in regular gametic mitoses.

When attention is directed to the highly inconstant and largely sterile species which Brainerd and Peitersen on the basis of their Mendelian segregation consider to be of hybrid origin, the situation becomes even clearer. For example, *R. andrewsianus* Blanchard, which is considered by Brainerd and Peitersen to be a hybrid between *R. alleghaniensis* and *R. argutus,* both good diploid species, with normal generative mitoses, we find a triploid form similar to that derived by the crossing of diploid Oenotheras or diploid species or varieties of Morus. Poorly developed pollen and a meiotic mitosis resembling that of known hybrids are both exemplified by this obviously hybrid species. In *R. jeckylanus* Blanchard, which Brainerd and Peitersen consider to be a cross between *R. argutus* and *R. frondosus,* together with bad pollen, we find hexaploidy and irregular mitoses such as are typical of hybrids. The same statement stands for *R. frondosus* Bigelow; *R. plicatifolius* Blanchard, a hybrid between *R. flagellaris* and *R. frondosus,* according to the results of Brainerd and Peitersen, is pentaploid and with this polyploid outfit of chromosomes unites a high degree of sterility and hybrid-like heterotypic and homotypic divisions. The same statement holds true for *R. hispidus* L., in which the pollen is ninety per cent. imperfect and the chromosome equipment pentaploid. It is in a variety of this species probably of hybrid origin that Dr. Longley has found the largest number of chromosomes yet exemplified by the genus Rubus, namely, fifty-six or the octoploid equipment.

It would be possible to continue almost indefinitely in the case of Rubus, to point out the highly significant parallelism and agreement of the systematic, genetical and morphocytological evidence as to the hybrid origin of many of the species of the genus. This striking agreement can not be neglected in forming any hypothesis of the origin of species. Nothing can be clearer in the genus under discussion than that polyploidy has arisen in connection with hybridization in nature and that this polyploidy is both of the artioploid and the perissoploid type. In Rubus, accordingly, there is convincing evidence both of the origin of species by crossing and equally of the development of polyploidy as an accompaniment of heterozygosis. If this be admitted, and it is not easy to see how the force of so many harmonizing different lines of evidence can be denied, the same explanation with a high degree of probability can be asserted of the origin of species and of polyploidy in Rosa and Crataegus, on the basis of the cytological investigations of Täckholm and Longley, concerning which we have as yet no genetical evidence. On account of the greater age which must be reached before the gonads are developed, these genera are not well suited for genetical investigation, within reasonable limits of time.

This situation in the case of Rubus is further interesting because of the light which it obviously throws on the conditions in such forms as *Oenothera lamarckiana* and other species of that genus, which in the past two decades have supplied abundant material for the investigation of the process of mutation, so called. As is well known, *O. lamarckiana* and other species of the genus throw in cultures, types, which were first designated by DeVries, and others, Gigas, Simigigas and Nanella types. Lidforss was successful in finding similar offshoots from known hybrids of Rubus, a situation obviously of the greatest importance in connection with the interpretation of the so-called process of mutation. The various species of Oenothera show the same morphological characteristics as do hybrid species of Rubi, namely polyploidy, irregular meiotic mitosis, polyspory, polycary, lagging chromosomes and chromosomes eliminated into the protoplasm at the time of formation of the daughter nuclei. All these abnormalities belong together and all point towards hybridism as the fruitful cause of the origin of new species. In Rubus the evidence is abundant, many sided and convincing, and this genus will accordingly serve convincingly to illustrate for the present the origin of new species in connection with crossing and resulting polyploidy. There can be little doubt that Datura and Drosophila will be brought under the same rubric in due time. It is interesting to note that Tower in his work on hybrid potato-beetles found that when the members of the uniform hybrid race were segregated they immediately began to "mutate" and to give rise to apparently new species.

Time does not permit me to follow further this interesting theme, but I venture to urge in conclusion that it is important that zoologists and botanists make greater effort to view the process of the origin of species on the basis of the coordinated, comparative and experimental study of the species of large genera rather than on that of the intensive genetical investigation of such isolated and interesting freaks as *Oenothera lamarckiana, Datura Stramonium* and *Drosophila melanogaster.* The purely experimental method appears to have become almost an obsession in biology during the past two decades, and geneticists, in the language of Professor Wilson, have got too far away from their morphological base. It may be safely predicted that when this many sided and integral study of species becomes general we shall have much clearer and more logical views than at present. In this connection the great importance of the epochal discoveries of Rosenberg on the cytology of hybrids will be realized.

SOME REMARKS ON SPECIES AND CHROMOSOMES

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THE aim of taxonomy, taken in its higher sense, is to describe the forms (species) of the animal and vegetable kingdoms as completely as possible, with the object of helping to explain their relationship and phylogeny, or, in other words, to try to understand their origin and evolution.

Any help to a fuller description of these forms I therefore consider an advance, and I agree with Dr. R. R. Gates that the stating of the number of chromosomes of a form (species) is one more element in its description—and an important one. But I must maintain that the chromosome number does not make the description complete, as there are many other elements of description which are also important.

When Linnaeus described his species, he was satisfied with one or two brief statements of morphological characters. Later we employed much fuller descriptions, and we may eventually take ecological and physiological characters also into consideration, as the microbiologists already do. Some time ago anatomical characters were in vogue, and cytology may be considered as a modern phase thereof, especially the counting of the number of chromosomes for each species. To take a rather wider perspective, the pH-behavior of a form is another modern phase of study which of course also has its importance in a full description of the forms in question.

Now these different features are all merely of a descriptive character, as is also the affirmation of polyploidy in a genus, a discovery which has considerably increased our knowledge of the supposed relationship within certain genera and families. But in being able to understand the origin of new forms (species) and, furthermore, in being able to produce such new forms, we have not advanced very far.

No doubt hybridization plays an important part in the production of new species, and the cytological behavior in hybrids has shown us how the chromosomes arrange themselves. Sometimes we get a stable arrangement, and then the hybrid may be considered a new form (species). Sometimes the arrangement is unstable, and the hybrid splits up. Hybridization does not, however, according to my opinion, help us beyond a certain point, and seems to be most effective in the so-called polymorphous genera. Forms (species) seem to arise in other ways still; we may call them mutations, which only means that we do not know anything about their origin.

What we want to find out—and we have up to the present not many hints to show us the way—are the causes which produce new forms, and still more, we hope in the future to be able to produce the new forms ourselves by inducing changes in the present ones. These changes may be changes in the number of chromosomes, showing themselves also in the alteration of other characters, or it may be changes which, without altering the chromosome number, are visible as new morphological or physiological characters of the form. Until we have compassed this end, we have not reached our ultimate goal—namely, a full knowledge of the origin of species.

ANIMAL PARTHENOGENESIS IN RELATION TO CHROMOSOMES AND SPECIES

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THE subject of this contribution relates to a small facet only of the subject under discussion at this gathering.

It is a small facet because the data at our disposal are meager, but, nevertheless, as no one yet has referred to it in this symposium there is justification for inviting your brief attention to it. If its discussion appears somewhat too speculative it may be asked—surely speculation is one of the *raisons d'être* of these intersectional meetings.

Lotsy, as is well known, champions the theory that hybridization between species gives rise to new species, but it is perhaps not so well known that hybridization, according to Ernst, may give rise to parthenogenesis. This idea naturally follows: Is there any connection between the emergence, through hybridization, of new forms and the emergence, through hybridization, of a new method of propagation of these novelties? Stated in cytological language, have the cytoplasmic and chromosomal constitution of the oocytes of a new hybrid form or the reactions between the two complexes entering into the composition of these oocytes endowed these reproductive cells with the faculty of segmenting and developing without the stimulus of fertilization?

So far as is known among animals, in a group in which thelytokous parthenogenesis is of long standing, as for instance among saw-flies (Tenthredinidae), there is maintained a stereotypy of form. But if we are dealing with a newly produced hybrid which is parthenogenetic we must not expect it, by its very nature, to be parthenogenetically capable of producing offspring diverse both morphologically and sexually; that is unless the oocytes of the F_1 generation hybrid be in such an unstable state chromosomally, and possibly cytoplasmically, that the "simpler" and "short-cut" asexual method of reproduction, in which the complications from union with the male gamete are dispensed with, still does not prevent anomalous meiotic behavior which finds expression in novel forms. Hybridization has even been stated to be a cause of parthenogenesis and such would postulate, as Winkler contends (1), that breeding experiments and cytological work had demonstrated both the parent species to be parthenogenetic. Considering the exacting nature of such experimentation it is not surprising that a satisfactory demonstration of hybridization as a cause of parthenogenesis does not exist either among plants or animals, though the following isolated zoological example is a close approach. From the botanical evidence of to-day the idea that hybridization is an occasion, but not a cause of the appearance of parthenogenesis, is acceptable, the species crossing, as it were, releasing a latent or slight tendency to parthenogenesis in one or both parents as well as enabling it to develop more fully and to be exploited more freely. However, whatever be true in either or both these views, it makes no difference to the discussion on the rôle of parthenogenesis in the segregation of new and diverse forms, or the repetition, maintenance and fixation of new stereotyped forms, from parthenogenetic F_1 hybrids. So far as I am aware the following results of Harrison[1] (2) afford the only zoological evidence of this thesis comparable to that obtained from the botanical side.

By crossing the two species of moths, *Tephrosia bistortata* and *T. crepuscularia* F_1 hybrids of intermediate character were obtained and one of the females laid parthenogenetically. The parthenogenetically laid eggs yielded an F_2 generation of one female of a more or less nondescript character and two males, one of which was as dark as any specimen reared from normal crossings, while the second was a new form, pale and peppered in appearance. None, however, was comparable with the normal F_2 generation obtained sexually. Beyond this the experiment did not go. Other data relating to the experiment are as follows. The proportion of F_1 hybrids which actually laid parthenogenetically was small. Further, compared with conditions in the parents, the number of parthenogenetic eggs laid was fewer and, moreover, the proportion of eggs which hatched was less. Again, the larvae were not easy to rear and their mortality was heavy. Also, there was evidence of anomalous chromosome behavior in the F_1 hybrids. Lastly, repeated and prolonged experiment has not revealed any parthenogenesis in either of the parent species.

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[1] These results were obtained as a by-product in other genetical experiments, and for other data cited in this contribution and not published in his paper I am indebted to my friend and colleague, Dr. J. W. Heslop Harrison. For the views expressed on these data the present writer, of course, is alone responsible.

We may now discuss the significance of such results. Firstly, whilst it seems possible that parthenogenesis was not inherited from either of the parent species we must await further experimental results in lepidoptera and other animals before asserting that hybridization in these moths evoked parthenogenesis *de novo.* But at least it seems reasonable to conclude that the hybridity and the parthenogenesis were correlated. Have we here a case in which the reactions between the parental chromatins in the parthenogenetic egg have been so violent as to cause parthenogenetic development or, at least, to stimulate anew any inherent latent capacity for parthenogenesis? In view of the well-known results obtained by simple physical and chemical agencies used in artificial parthenogenesis we can not dismiss the possibilities consequent upon the more intimate and vital reactions between different chromatins. That the latter has occurred in this experiment seems evident from the anomalous chromosome behavior. Secondly, the parthenogenetic hybrid, though intermediate compared with the parent species, did not breed a stereotyped race but, by segregation of characters, new forms arose which were diverse in appearance and in sex. By further breeding from this F_2 generation, whether by sexual or parthenogenetic means, new sexual or / and parthenogenetic races are theoretically possible. As the facts stand, however, we have in this case a unique zoological example of hybridity operating by parthenogenesis to produce new forms; that is, assuming the chromosomes to be the physical basis of heredity, the reactions between the two specific parental chromosomes have given expression to a different mode of inheritance, *viz.*, the parthenogenetic by means of which new phenotypical and genotypical forms have been propagated.

Apparently the only other zoological work which illustrates correlation between parthenogenesis, chromosomal changes and character changes is that of Artom (3). This worker found that the Cagliari race of the brine shrimp *Artemia salina* was bisexual and had a diploid chromosome number of 42 while the Capodistria race was parthenogenetic with a tetraploid chromosome number of 84. But, in addition, the parthenogenetic race showed a somatic difference—the epithelial cells of the mid-gut (endodermal) were larger in size and fewer in number. As is generally admitted for animals, species now parthenogenetic have evolved from a former bisexual condition and such appears to be the case in *Artemia salina.* Hence hybridization and its sequelae of cytological disturbances are ruled out. Rather we are dealing with a case in which the tetraploid nucleus of the parthenogenetic form, in its chromosome content —not in its quality as in the hybrid oocyte—has some relation to the character of the soma. In other words whatever the mode of origin of the tetraploid condition and whatever the causal relation, if any, between tetraploidy and parthenogenesis, we see here a case in which parthenogenesis is utilized as a mode of maintaining a race possessing a form which differs genotypically from the form of the bisexual and parent race.

SUMMARY

(1) Zoological evidence, admittedly meager in amount, concerning the correlation of parthenogenesis with the emergence and propagation of new forms, is presented from the work of Harrison on hybridization of the moths *Tephrosia bistortata* and *T. crepuscularia* and Artom on tetraploidy in the brine shrimp *Artemia salina.*

(2) Harrison's results which show that crossing between the two species has resulted in the production of F_1 hybrids intermediate in character and parthenogenetic in sex which, in turn, have produced a small F_2 generation of new types of both sexes by character segregation, are regarded as illustrating that among animals it is possible that hybridity and correlated parthenogenesis may be responsible for the emergence of new forms.

(3) Artom's results are interpreted as illustrating that parthenogenesis has been closely correlated with the emergence, and has been responsible for the maintenance of the more recently derived tetraploid and genotypically different Capodistria race of *Artemia salina.*

REFERENCES

(1) Winkler, H. Verbreitung und Ursache der Parthenogenesis in Pflanzen und Tierreich, p. 141 *et seq.* Jena, 1920.

(2) Harrison, J. W. Heslop. "The Inheritance of Melanism in the Genus *Tephrosia* (*Ectropis*) with Some Considerations of the Inconstancy of Unit Characters under Crossing." *Jour. of Genetics,* Vol. X. No. 1, July, 1920.

(3) Artom, C. Le basi citologische di una nuova sistematica del genere Artemia. *Archiv für Zellforschung* 9. Band, 1. Heft, 1912.

APPENDIX

Since writing the above, the writer has just obtained an interesting corroborative result from material kindly supplied by Dr. Harrison, particulars of which are as follows. From a certain experiment in 1923, related in some detail in *Nature,* August 18, 1923,[2] Dr. Harrison obtained in February, 1924, a melanic male of *Tephrosia bistortata,* melanism being recessive. This male was crossed with a type (white) *T. crepuscularia* female in February, 1924, and the resulting F_1 hybrid generation of May-June, 1924, were of both sexes and light like the female parent. One of these light colored hybrid females laid 200 to 300 unfertilized eggs though only 6 larvae emerged. These larvae, newly hatched, were handed to me and reared on knotgrass (*Polygonum aviculare*) but only two reached the pupal stage; the others, for some unaccountable reason, died. The two pupae wintered from July, 1924, and in January, 1925, precise date uncertain, one of the moths emerged but, unfortunately for further experiment, was dead when discovered. It was a melanic female. The second pupa on examination was found to be dead but it had developed sufficiently far to show that it was a melanic male. The experiment may be set out thus:

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[2] Garrett, F. C., and J. W. H. Harrison. Melanism in Lepidoptera and its possible induction. *Nature,* Aug. 18, 1923, p. 241.

Melanic *T. bistortata* ♀ ; ww ×
Normal white *T. crepuscularia* ♀ ; WW
|
White hybrid ♀ ; Ww; parthenogenetically has given rise to
|
Two pure recessive melanics; 1 ♂ and 1 ♀ ; ww.

It is curious and interesting that the two specimens which survived to an identifiable stage were melanic recessives when the chance of such forms appearing was only one in four even under ordinary Mendelian conditions.

Dr. Harrison also informs me that despite repeated attempts since the specimens mentioned in the above contribution were initiated—six years ago—he has never again obtained parthenogenetically laid eggs until May, 1924.

THE PHYSIOLOGICAL SIGNIFICANCE OF CONJUGATION AND ENDOMIXIS IN THE INFUSORIA[1]

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THE first serious attempt to give a comprehensive survey of the Protozoa is afforded by the studies of Otto F. Müller made during the latter part of the eighteenth century, though it is true that Dr. John Hill in his "History of Animals," published in 1752, stated that "their want of magnitude does not exclude them from their rank among animated beings . . . I have arranged them into a regular method, and given them denominations"—and one denomination he coined was Paramecium. But Müller, profiting by the microscopical studies in the interim, as well as by the establishment of the Linnaean system of classification, produced in his Animalcula Infusoria the actual base line for future investigations. Our present interest, however, in Müller's work is due to the fact that he rightly interpreted pairs of Infusoria as undergoing a sexual process and not as dividing longitudinally. The latter, strange to say, was the view of his successors down to 1858, when Balbiani published his observations on the existence of sexual reproduction in the Infusoria.

Balbiani thought that the nucleus and micronucleus became transformed into sexual organs—the macronucleus producing eggs, and the micronucleus, sperm—and he thereby obscured a minimum of fact with a plethora of imagination. Nevertheless, with Balbiani's work as the point of definite departure, the problem at the hands of Bütschli and Engelmann in 1876 was placed well upon the road toward its present-day development. These authors established the dimorphic nature of the nuclear apparatus of the Infusoria and the formation of a new macronucleus from the micronucleus during conjugation, though it remained for a series of workers during the eighties of the last century—notable among them being Balbiani, Maupas and Richard Hertwig—to observe the important cytological features of conjugation, including synkaryon formation, in the Protozoa.

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[1] Lecture delivered at the Woods Hole Marine Biological Laboratory, July 18, 1924.

But both Bütschli and Engelmann did more than supply cytological data: they offered the first concrete suggestions in regard to the significance of fertilization. Bütschli believed it to be a process of rejuvenation, and Engelmann one of reorganization: a difference in details, but an agreement in fundamentals. Both thought that continued reproduction by division tends to slow down through loss of vitality, and that fertilization restores the pristine vigor of the cells. And thus the so-called "dynamic" interpretation of fertilization phenomena in the Protozoa arose.

These pioneer studies of fertilization in the Protozoa were made during and, it is probably fair to say, under the stimulus of the classic period of cytological advance. Bütschli and Auerbach, Oscar Hertwig and Fol, Flemming, van Beneden and Boveri were establishing the essential features of mitosis, maturation and fertilization in metazoan eggs which gradually led to the full realization of the genetic continuity of the germ nuclei foreshadowed a generation before. And this, in turn, formed the indispensable prelude to the elucidation of the chromosome mechanism which cytologists have supplied to geneticists as the physical basis of heredity.

Thus was added content to the view, emphasized two centuries before by Harvey and modernized by Oscar Hertwig and Minot, that fertilization supplies a stimulus to egg development, and to the equally ancient and probably more important one that fertilization affords the basis of biparental inheritance in all sexually reproducing organisms.

The answers to several fundamental physiological problems have been sought in the study of the Infusoria, which may be stated in the form of two general questions:

(1) Do these organisms possess the power to reproduce indefinitely by division, under favorable environmental conditions, without recourse to conjugation or to endomixis?

(2) In cultures which are exhibiting lowered vitality, does either conjugation or endomixis restore the normal metabolic condition and "rejuvenate" the organisms?

We shall consider these and attempt to present definite answers, but it is unnecessary to state that since there are always at least two sides to every question, impartial as the reviewer wishes to be, the personal equation must perforce find expression.

The first consistent investigation of the power of the Infusoria to reproduce indefinitely without recourse to conjugation is afforded by the elaborate studies of Maupas in which he presented a wealth of data and a series of interpretations that for more than a decade were taken at their face value and believed to prove that the life history runs in cycles. In brief, a long period of multiplication by cell division is succeeded by an epidemic of conjugation, which inaugurates a new cycle, and is comparable in its physiological aspect with the period of sexual maturity in the metazoa. If conjugation does not occur, the race rapidly degenerates and dies, and accordingly conjugation counteracts the tendency to senile degeneration and causes rejuvenescence.

Now to-day we are asking whether Maupas's data show what he says, and whether he says what he means! This is not merely of historic interest because his hundreds of pages filled with observational and experimental details have had great weight.[2]

Maupas definitely says, "My cultures have experimentally shown that these Protozoa do not escape the general law of senescence. They exhaust themselves and become old by the exercise of their functions, or, in other words, because of a necessity inherent in their structure and constitution.'' This, as Calkins points out, is exceedingly explicit and is the burden of Maupas's entire experimental study.

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[2] Calkins, "What did Maupas mean," AMER. NAT., 57, 1923.

But Maupas states that his records indicate that "the power of reproduction follows no such diminishing and parallel course. It is maintained almost intact even a long time after the other functions and the entire organism are shown to be greatly reduced by senile degeneration . . . the Infusoria, although feeble and much reduced in size, continue to divide with almost the same rapidity as at the beginning.'' In other words, Maupas apparently believed that progressive physiological and morphological degeneration is compatible with a normal division rate. As Calkins's analysis has shown, Maupas's own data do not indicate the biologically untenable conclusion that division rate, which subsequent investigation has amply demonstrated is a most subtle index of metabolism, is unaffected by degenerative changes.

So, although we can at least accept it as a fact that Maupas found progressive degeneration as cell division proceeded, we must reject his statement that the division rate was unaffected. His studies indicate cyclical change, both structural and physiological, during interconjugation periods. And thus the so-called cycle definitely entered the literature on the Infusoria.

Without minimizing the importance of the pioneer work of Maupas, it is nevertheless true that his method of culture was inadequate to the problem, and the great advance which paved the way for all more recent work was the adoption of the daily isolation method by Calkins when he took up the problem after Maupas's results had been accepted for over a decade. This method rendered possible careful control of the culture medium from day to day, accurate records of the rate of division and the positive elimination of conjugation. In brief, it afforded for study precise pedigree cultures of Infusoria.[3]

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[3] Calkins, "Studies on the life history of Protozoa. I. The life cycle of Paramecium caudatum," *Arch. f. Entw-mech.*, 15, 1902.

Calkins's investigations on the question of the cyclical nature of the life history of Infusoria began in 1901 with Paramecium caudatum and have been continued on various species, culminating in an extensive analysis of the life history of Uroleptus mobilis which is still in progress. His answer to the question under consideration is that the "physiological processes of metabolism are not capable of unlimited activity . . . there is a progressive weakening of metabolic vigor from an optimum shortly after conjugation." "Such weakened protoplasm, if not allowed to conjugate, inevitably dies, as does the somatic protoplasm of metazoa." "Natural death from old age . . . is actually inherent in protoplasm as little differentiated as in these single-celled animals."[4]

But other workers, following the lead and using, in general, the technique of Calkins, have reached other results and conclusions. Enriques has bred cultures of several Infusorians, notably Glaucoma pyriformis, for nearly 3,000 generations, without signs of lowered vitality. At New Haven, Paramecium aurelia has been carried for some fifteen years and more than 10,000 generations, and, among others, Spathidium spathula for over a year, during which 1,000 generations were attained. Conjugation has not occurred in the direct line of any of the cultures. Similar results have been attained by Baitsell on Pleurotricha lanceolata and Oxytricha fallax, by Mast on Didinium nasutum and by Dawson on Oxytricha hymenostoma.

It is highly significant that the longevity of Infusoria —the so-called life cycle—has been gradually though consistently prolonged year by year as pedigree culture technique has been perfected. Even such relatively ubiquitous forms as Paramecium aurelia and caudatum, which are able to survive temporarily under very diverse conditions, gradually succumb to prolonged subjection to media which at first seem entirely suitable. Ample evidence of this is afforded by the history, during more than threescore years, of the study of Paramecia cultures.

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[4] Calkins, "Uroleptus mobilis, II, Renewal of vitality through conjugation," *J. Exp. Zool.*, 29, 1919.

Quite a similar history has been briefly recapitulated at New Haven in the study of Spathidium spathula during the past four years. The majority of the cultures showed what heretofore would have been interpreted as inherent "cycles" which culminated in death unless conjugation was permitted, but the drift toward longer life of certain cultures as the experiments progressed, and finally the attainment, as just mentioned, of more than a thousand generations with every indication of the potentiality to continue to divide indefinitely, can, we believe, be reasonably interpreted in but one way.[5]

The only species extensively bred which still proves refractory is Uroleptus mobilis. The extreme rarity of this species may reasonably, we believe, be attributed to peculiar conditions of life demanded, and its cycle to the fact that some crucial factors of the milieu have thus far not been supplied. If this is not the proper interpretation of the cyclical character of the Uroleptus life history, then this form certainly suffers a limitation which is not shared by the other species investigated.

To one question, then, we believe an unequivocal answer has been given—the Infusoria in general can, under favorable environmental conditions, reproduce indefinitely by division without recourse to conjugation. The cycle is a phantom which is slowly but surely receding toward the vanishing point, as the special requirements of particular organisms are being established.

From the study of various species of hypotrichous Infusoria as well as from the long continued Paramecium aurelia culture at New Haven, it was found that minor periodic fluctuations of the division rate occur, from which recovery is autonomous. These fluctuations were termed rhythms and contrasted with the so-called

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[5] Woodruff, "The present status of the long continued pedigree culture of Paramecium aurelia at Yale University," Proc. Nat. Acad. Sci., 7, 1921.

Woodruff and Moore, "On the longevity of Spathidium spathula without endomixis or conjugation," Proc. Nat. Acad. Sci., 10, 1924.

"cycle," which comprises a varying number of rhythms and, according to Calkins, ends in the death of the race if conjugation or artificial stimulation is not resorted to.[6] An intensive study of rhythms was made in Paramecium aurelia which showed that subjection of the culture to the most constant environmental conditions failed to eliminate the rhythms, but, instead, they appeared still more pronounced. It was also found from a study of the temperature coefficient of the rate of division that this is influenced by temperature at a velocity similar to that for a chemical reaction, except when the rhythms interfere. Thus it became apparent that there are inherent rhythmical changes in the phenomena of the cell which produce slight fluctuations in the rate of division.[7]

Daily cytological study of pedigree Paramecium aurelia and caudatum showed that the rhythms in these species are coincident with a nuclear reorganization process which was termed endomixis. It will be recalled that an essential difference between endomixis and conjugation is the absence of the third micronuclear division, which in conjugation forms the gametic nuclei, and, of necessity, the non-formation of a synkaryon.[8]

This process of endomixis—or, if you will, parthenogenesis, though we believe the non-committal term preferable at the present stage of our knowledge—has also been found in one other species of the genus Paramecium, P. polycaryum; Calkins has found it in Didinium nasutum and Uroleptus mobilis, and Moore in Spathidium spathula. Fermor described an internal reorganization process during encystment in Stylonychia pustulata which, however, involves a nuclear fusion and is, therefore, not endomixis but autogamy.[9]

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[6] Woodruff, "An experimental study on the life history of hypotrichous Infusoria." *J. Exper. Zool.*, 2, 1905.

[7] Woodruff, "The influence of general environmental conditions on the periodicity of endomixis in Paramecium aurelia," *Biol. Bull.*, 33, 1917.

Woodruff and Baitsell, "The temperature coefficient of the rate of reproduction of Paramecium aurelia," *Amer. J. Physiol.*, 29, 1911.

[8] Woodruff and Erdmann, "A normal periodic reorganization process without cell fusion in Paramecium," *J. Exp. Zool.*, 17, 1914.

[9] Fermor, "Die Bedeutung der Encystierung bei Stylonychia pustulata," *Zool. Anz.*, 42, 1913.

The discovery of endomixis in Paramecium aurelia and caudatum coincident with rhythms which are due to intrinsic and not external factors naturally led to the interpretation of endomixis as the phenomenon involved—a rhythm thereupon being regarded as the outward physiological expression of the underlying cytological phenomena of endomixis. Furthermore, it was shown in Paramecium aurelia and caudatum that cessation of endomixis is soon followed by the death of the culture. This affords the best possible proof that a periodic occurrence of endomixis is indispensable for the continued life of these species.

So far as Paramecium aurelia and caudatum are concerned, the rhythm may perhaps be considered as an expression of a sort of temporary "senescence" for which is provided an internal, automatic antidote in the form of endomixis. But I would emphasize that this is a case of trying to force new wine into old bottles in order to save an idea. One certainly must grant that the original idea of senescence in Infusoria is far removed from this subtle, automatically arrested type which rhythms may indicate. To shrink the cycle to the rhythm is to annihilate the cycle.

But we are not interested in an academic discussion of terms—the fact remains that still more recent work has shown [illegible]at in other species of Infusoria endomixis does not occur during the free life of the cell, though rhythms, as originally defined, are present. This is the case in Spathidium spathula—endomixis occurring, as Moore has shown, during encystment; and in Paramecium calkinsi, in which endomixis, as demonstrated by Spencer, does not take place at all. In other words, endomixis occurs at the low point of the rhythms in Paramecium aurelia and caudatum, and though, from the very nature of the process, it accentuates the drop in the division rate, nevertheless it is not the underlying factor of rhythms after all—for rhythms exist *without* endomixis in other species.

Such being the case, the whole problem takes on another aspect: we have two phenomena to consider—rhythms and endomixis. For the moment, however, we are seeking a definite answer to the specific question whether Infusoria can reproduce indefinitely by division without endomixis. It is clear that Paramecium aurelia and caudatum can live indefinitely without conjugation, but that suppression of endomixis inevitably is followed by death, and accordingly endomixis is indispensable in the life history of these species. But Enriques states that endomixis did not occur in his long-continued Glaucoma culture, and at New Haven it has been shown that Paramecium calkinsi, which does not undergo endomixis, apparently can reproduce indefinitely by division, and that Spathidium spathula, which undergoes endomixis solely while encysted, can reproduce indefinitely by division without encystment and, therefore, without endomixis.[10]

Accordingly, although the suppression of the endomictic process while in progress in the free life of Paramecium aurelia and caudatum is fatal, the undoubted fact that in other species of Infusoria it may be eliminated by preventing its onset (*e.g.*, by preventing encystment) and in still other species it apparently does not occur at all, forces us to the definite conclusion that endomixis is not a fundamental, indispensable factor in the life history of the Infusoria—that they can reproduce indefinitely by division under favorable environmental conditions.

In brief, the time-honored question whether the Infusoria degenerate and die when conjugation is prevented has, we believe, been answered unequivocally in the negative, and likewise, the very new question whether Infusoria in general degenerate and die when endomixis does not take place has also been answered unequivocally in the negative. Therefore, not only is synkaryon formation unnecessary, but a periodic reconstitution of the nuclear apparatus from the micronucleus is unnecessary. All the evidence cumulatively points to the conclusion that the zygotic nucleus constituted during syngamy and the nucleus constituted during endomixis in the Infusoria are adequate for indefinite division of the cell under favorable environmental conditions.

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[10] Enriques, "Duemila cinquecento geneazioni in un infusorio, senza coniugazione nè partenogenesi, nè depressioni." R. Acad. Sci. Bologna, 1916.

Moore, "Endomixis and encystment in Spathidium spathula," *J. Exp. Zool.*, 39, 1924.

Spencer, "Studies on Paramecium calkinsi," *J. Morph. and Physiol.*, 39, 1924.

Having eliminated, to our satisfaction at least, amphimixis, endomixis and the cycle we still have the rhythm. To what are rhythms due? Defined from a study of the life history of hypotrichous Infusoria in 1905 as "a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous" they have since then been in evidence in every culture of Infusoria whose division rate has been published. Endomixis in Paramecium aurelia and caudatum was believed to offer the solution, but it is evidently not the cause of the rhythms—though it accentuates them in these species—since our belief that endomixis would be found during the rhythms of every species has not proved to be correct. We are forced, therefore, to fall back for the present on our general statement of twenty years' standing—"due to some unknown factor in cell metabolism."

But there is one possibility which, remote as it is, must not go unmentioned. Rhythms in Infusoria synchronous with endomixis may not be the same phenomenon as rhythms in forms which do not undergo an endomictic process. Although the term was originally applied to fluctuations in the fission rate of species which subsequent work has shown do not have endomixis, and then applied to fluctuations in the rate of Paramecium aurelia which later work has shown does undergo endomixis, it is a fact that the most extensive attempts to eliminate these fluctuations by constant environmental conditions were made on Paramecium aurelia before endomixis was known. An equally intensive study of a form without endomixis might show that the rhythms can be eliminated, although Spencer has already carried on a series of cultures of Paramecium calkinsi for this particular purpose which makes it almost certain that the rhythms are inherent.

We have considered the question whether conjugation is a necessary factor in the life history of the Infusoria and have answered it in the negative. But, obviously, because conjugation is not necessary under favorable environmental conditions, it does not follow that it is not necessary under other conditions—that it does not have a dynamic function which is brought into play when the organism meets various vicissitudes of its environment.

In opposition to the prevailing view based chiefly on the work of Bütschli, Maupas insisted that conjugation does not increase the rate of reproduction: it merely wards off senile degeneration when it occurs before this is far advanced. Richard Hertwig from very limited experimental evidence reached similar results, except that he found after conjugation the division rate was *lower* than before—and so he interpreted the rejuvenating effect of conjugation as due to a repression of division energy.

However, the work which is significant to-day begins with Calkins's studies carried out twenty years ago on Paramecium caudatum. In degenerating pedigree cultures of this organism, he found that so-called rejuvenescence occurs after exogamous or endogamous conjugation, but only in a small percentage of cases, for only a few unions result in continued life of the exconjugants. These failures, he thought, indicate that only certain combinations are capable of yielding fertile results, especially in view of the fact that he obtained a high percentage of viable conjugants from wild Paramecia. From further work on the same organism he concluded that conjugation between two cells results in the complete reinvigoration of all activities, both physiological and germinal.

So the problem stood when Jennings in 1913 published his extensive study on the effects of conjugation in Paramecium, in which he marshalled the data from Maupas and Hertwig to support his own brilliantly attained result, from experimental and biometrical analysis, that "the average rate of reproduction is less after conjugation than before." That is, when he took, for example, two sets of animals of the same stock and history, both ready to conjugate; permitted one set to conjugate and prevented the other, he found that those which had conjugated divided thereafter on the average less rapidly than the others. In most cases the rate of fission was very considerably greater in those that had not conjugated, and in no cases did the conjugants have a higher rate of fission. Furthermore, as a rule mortality was much higher, under the same conditions, among animals that had conjugated than among those which had been prevented from conjugating—regardless of whether the animals were unrelated or were members of the same race. So Jennings naturally asked: "Is it not time that the statements or implications that in the Infusoria conjugation results in increased reproduction should disappear from the literature of science?" And four years later Mast's studies on Didinium nasutum led him to agree that conjugation has no appreciable effect on the death rate, fission rate or variation in the fission rate.[11]

In 1917 Calkins resumed his study of the problem, employing a pedigree culture of the hypotrich Uroleptus mobilis. Carrying more than thirty series from exconjugants, he found that there was a progressive weakening of metabolic vigor from an optimum shortly after conjugation. This progressive weakening he attributes, as we have stated, to inherent senescence, while we would attribute it, as we have our earlier results with Spathidium, to extrinsic causes. However the condition arose, the significant fact for the moment is that the animals were transformed from a condition of metabolic weakness to a condition of optimum metabolic vigor by the process of conjugation. Animals which were not allowed to conjugate, under the conditions of the experiment, died.

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[11] Jennings, "The effect of conjugation in Paramecium," *J. Exp. Zool.*, 14, 1913; "Life and Death, Heredity and Evolution in Unicellular Organisms," Boston, 1920.

Now since Jennings showed on a large scale in his studies "that conjugation brings about great variation in vitality and reproductive power" in Paramecium, it is perfectly reasonable to regard single examples of increased fission rate following conjugation as one of the rare extreme variants. But Uroleptus shows almost without exception stimulation following conjugation, so that this explanation is no longer tenable; and Calkins in these experiments has certainly supplied the first apparently unassailable evidence that conjugation is followed by renewal of vitality as indicated by increased reproductive power.

Before 1920 our studies were directed to the question: What happens when conjugation is prevented? But then we turned to the question: What happens when conjugation occurs? Spathidium spathula was selected for the work after a long search for favorable material because it affords the several features which are essential for the successful attainment of the desired data: sufficient size to render practical the daily isolation of large numbers of animals; the non-occurrence of endomixis in free living stages; and, most important of all, the frequent onset of epidemics of conjugation between pedigree animals so that non-conjugant and conjugant lines can be secured and compared at crucial periods. Conjugation could be obtained almost at will by allowing a few animals to remain on a culture slide for a day or two without an adequate supply of Colpidia, which is the staple diet of Spathidium. Incidentally, it may be mentioned that Moody's experience with Spathidium was quite different in that no evidence of ability to conjugate was evident, apparently because it was an amicronucleate race.[12]

[12] Woodruff and Spencer, "Studies on Spathidium spathula, I, The structure and behavior of Spathidium," *J. Exp. Zool.*, 35, 1922; "II, The significance of conjugation," *J. Exp. Zool.*, 39, 1924.

All the animals used in our studies were descendants from a single "wild" animal collected at New Haven. Standard beef extract was employed as the basic culture medium, and to this was added, as used, Colpidia raised in flasks of hay infusion. All the cultures directly compared were bred simultaneously under identical culture conditions.

The general method of conducting the pedigree lines may be briefly stated. From the original animal four lines were carried as the stem culture. Animals left over at the daily isolation were kept as stock, or allowed to conjugate or encyst. From time to time pairs of conjugants were isolated and their progeny established as "new" pedigree groups of cultures from the stem culture or its derivatives. Usually these groups comprised the progeny of two pairs of conjugants, though in a few cases the progeny of other pairs were also carried. In every instance the culture derived from a single exconjugant comprised four lines of animals and the average daily division rate of these four lines, again averaged for five-day periods, forms the basic data. At certain periods nearly 200 isolations were made daily, so that the investigation involved the manipulation of upward of 100,000 pedigree animals.

Passing over the question of the existence of a normal, inherent cycle in the life history of Spathidium, which we have already answered in the *negative* (cf. p. 230), we may summarize the data in regard to the effects of conjugation in cultures exhibiting a lowering of vitality under laboratory conditions.

A total of 118 exconjugants contributed to the experimental results. Of these, 18 died without dividing—a positive result—death immediately followed conjugation. However, the proportion of deaths is so small that it is negligible from the standpoint of this study. It is lower than the exigencies involved in such experimental work would naturally supply when the isolations and manipulations necessary are taken into account. Indeed, the high viability of exconjugants is remarkable in comparison with that obtained by most investigators studying similar problems. In addition to the cases just mentioned, 6 other exconjugants need not be considered since they were lost by accident. Thus there remain 94 exconjugants which established cultures affording cogent data, of which 79 ran synchronously with their parent cultures for three or more five-day periods, and so supply definite evidence bearing on the question: Do the progeny of exconjugants characteristically exhibit a significantly different (higher or lower) rate of reproduction from that of their parent culture which has not so recently conjugated?

A study of the initial fifteen days of each of the 79 exconjugant cultures shows that 67 divided more rapidly and 12 less rapidly than their respective parents. The 15 other cultures, whose parental cultures died within the fifteen days, all actually divided more rapidly than their respective parents, so that they may with justice be added to the regular 67 plus cultures to give 82 cultures showing an initial increase in the division rate. However, it is immaterial whether the latter cases are included or not, so far as the conclusion which must be drawn is concerned. Obviously, the data overwhelmingly indicate that the rate of reproduction in the great majority of cases is accelerated after conjugation.

But the analysis thus far has involved merely contrasting the division rate of exconjugant and parent cultures on the basis of an increase or decrease of division rate, without considering the amount of difference. Clearly, before a conclusion is secure, it is necessary to take into account the chance variation which is inevitable in any experiment in animal breeding such as the present. The usual biometrical methods of analysis are inapplicable here because in each experiment the individual cultures must be treated separately, and judged in relation to the synchronous division rate of their respective parent cultures and also of their grandparental cultures (if still surviving), as well as in relation to other cultures.

But the difficulties are more fancied than real, for by any reasonable criterion the number of cases in which the exconjugants exceeded the parent is far and away beyond the limits of any probable error that statistical methods may formulate. We have adopted a difference of five divisions in three periods, or an average of one third of a division per day, as a significant variation. Applying this criterion to the data gives 57 exconjugant cultures exceeding their respective parent cultures; 6 exconjugant cultures below their parent cultures, and 16 which are within this limit of error. A similar analysis in which seven divisions in three periods is taken as the significant variation gives 50 plus exconjugants, 5 minus exconjugants and 24 neutral. But our experience in handling the hundreds of pedigree lines during this experiment has convinced us that seven divisions in three periods is without doubt unreasonably large, and this is obvious when it is realized that this represents a change in the average rate of nearly one half a division per day. Nevertheless, even with this ultraconservative standard, the data give an unequivocal answer: conjugation in the great majority of cases directly induces an immediate acceleration of the reproductive activity.

A further analysis of the data, which need not be made at this time, fairly establishes for this pedigree race of Spathidium that conjugation usually is even more effective in accelerating reproduction when the exconjugants are derived from slowly dividing parents than when they are derived from rapidly dividing parents.

Turning now to the later effects of conjugation, we have 81 cultures which afford available data. Of these, 76 exconjugant cultures exceeded their parental cultures in the total number of divisions attained during their synchronous life, and 5 exconjugant cultures underwent a smaller number of divisions. Obviously this indicates that following conjugation the rate of division is accelerated.

But again, this analysis does not take into consideration the amount of difference in division rate between exconjugant and parental lines, and the difficulties involved here in determining a variation which is significant are still greater than in the comparison, just concluded, for the rates during a definite period of fifteen days. For now we are dealing with differences in duration: *e.g.*, some cultures ran synchronously for three periods and others for more than a dozen periods. Probably we can not do better than to take again the average variation of one third of a division per day as the criterion of a significant variation. Judged from this standard, 60 exconjugant cultures exceeded their parental cultures in division rate during their entire synchronous life, 3 exconjugant cultures were lower in division rate, and 18 cultures were within the limit of error of our standard.

Since conjugation is the sole variable involved in exconjugant and parental cultures, it is evident that conjugation directly induces not only an immediate acceleration of reproduction, but also an acceleration which persists at least as long as the life of the parental culture. And it is significant that the data from the first three periods and also from the entire synchronous life are practically identical, since this shows that the initial acceleration is maintained in essentially equal intensity to the end.

The question now to be considered is whether the progeny of exconjugants characteristically exhibit an increase or decrease in longevity in comparison with their parent culture which has not so recently conjugated. Among the 70 cultures which afford cogent data, 54 exconjugant cultures continued to live and reproduce after the death of their respective parent cultures, 10 exconjugant cultures died before their respective parent cultures, and 6 exconjugant cultures died essentially synchronously with their respective parent cultures. This analysis includes the cases where the exconjugant cultures when dividing rapidly suddenly died out in a day without preliminary signs, because no criterion exists for positively determining that such deaths were "accidental." But since this fate befell more exconjugant than parent cultures, if these were omitted from the analysis the relative number of exconjugant cultures which survived their parent cultures obviously would be increased.

Thus the data presented provide a very definite answer. In the great majority of cases the exconjugant cultures outlived their parent cultures, and since the advent of conjugation is the sole variable involved, it is apparent that conjugation tends to increase the time duration of life as well as the rate of reproduction in Spathidium. Therefore, necessarily, the number of generations attained by the exconjugant cultures exceeds that of the parent after the origin of the former. In brief, under the conditions of these experiments, conjugation has a survival value in the life history of this organism.

This conclusion naturally leads to a consideration of the effect of conjugation on animals from cultures which are actually on the verge of extinction, in order to determine if conjugation has a direct and immediate survival value.

Four groups of cultures offer definite data. In each instance the progeny of the exconjugant survived while the parent culture died. And this evidence, direct as it is, has been amply corroborated in a very practical way by all the other work on Spathidium which for more than four years has been carried on at New Haven. Cultures about to die have been continued with exconjugants. It is clear, therefore, that conjugation, in the great majority of cases, has a direct survival value.

The relation of conjugation to the continuance of the life of the pedigree race from the parental to the F_5 generation under cultural conditions can be best illustrated by a specific example. From the parental culture at the thirtieth division the F_1 generation was derived; from the F_1 at the 104th division, the F_2 generation was derived; from the F_2 generation at the 289th division the F_3 was derived; from the F_3 at the 150th division the F_4 was derived; and from the F_4 at the 80th division the F_5 was derived. The latter died at the 300th division.

Thus, from the parental culture to the death of the F_5 generation the series of pedigree cultures extended from November 5, 1920, to November 5, 1921, exactly a year, and the series could, without doubt, have been continued indefinitely by the same procedure. During the year the actual number of "linear" cell divisions from the parental culture through the F_5 generation of this series was 953. Since, under the conditions of the experiment, the parental culture died after 234 divisions on February 11, 1921, it is obvious that the life of the series, by five "conjugations" was prolonged approximately nine months, and exactly 719 divisions. Data may be similarly analyzed for the various generations of other groups, but it is unnecessary to do so here.

In brief, nothing can obscure the fact that under identical culture conditions "new" cultures, derived from conjugating pairs in an "old" culture, consistently survived the parent cultures. This, it appears, is a further definite demonstration of the survival value of conjugation.

The gradual "building up" of the division rate of a series through successive "conjugations" is also readily shown by the division rate of the same series of cultures which we have just been considering. The parental culture started with an initial rate of slightly above four divisions per day which was never again attained throughout its life. The F_1 generation attained but never exceeded the division rate of its parent. The F_2 generation never attained the maximum of its parental and grandparental cultures. The F_3 generation derived when its parent was dividing less than one and one half divisions per day and rapidly approaching extinction, exhibited a restoration of division energy which reached three and one half divisions per day. However, this is below the initial maximum of the previous generations. The F_4 generation, derived when its parent was dividing approximately twice per day, immediately attained and maintained until discontinued a rate of nearly four divisions per day. This is almost as high as the maximum of the P and F_1 generations, identical with the initial maximum of the F_2 generation, and much higher than that ever reached by the F_3 generation. The F_5 generation, derived when its parent was dividing at an average rate of nearly four times per day, exhibited an initial rate of nearly four and one half divisions daily, the grand maximum for the entire series.

Granting that the series selected as an example is the most striking case the data afford, the fact remains that the other cultures, directly involved in the specific experiments to "build up" the division rate to a maximum equal or better than that ever exhibited before by this pedigree race of Spathidium, consistently show a similar cumulative effect of conjugation. Accordingly, all the data involved demonstrate that it is possible by successive "conjugations" progressively to increase, within limits, the maximum division rate.

Throughout all these experiments the animals employed were descendants from the one "wild" Spathidium isolated to start the stem culture, and therefore each generation (F_1 to F_5) in the series was the product of close inbreeding. As a matter of fact, the two animals which conjugated were in no instance more than eight divisions removed from a common ancestor. Since, as has been shown, conjugation effects an equal, if not greater, acceleration of the reproductive rate during the later experiments, it follows that continued close inbreeding, at least to the F_6 generation, does not significantly alter the typical physiological effect following conjugation at the F_1 generation. But in order to compare exconjugant cultures arising by such close inbreeding with similar cultures arising from the crossing of distantly related animals, an attempt was made to cross animals which were separated by a large number of generations. The difficulties involved have thus far resulted in only one experiment out of over four hundred being satisfactorily carried through to completion. The animals mated were actually 982 divisions removed from a single common ancestral animal.

This culture and the necessary control cultures, involving 44 animals whose division rate was recorded daily for 25 days, showed no significant difference in rate of reproduction between the progeny of conjugants which were not more than eight divisions (about 48 hours) removed from a common ancestor and the progeny of the cross-conjugants, the members of which were removed 982 divisions (185 days) during which five "conjugations" were interpolated. Although no special weight should be given to this result, so far as it goes it is in accord with the data in regard to inbreeding from our other cultures.

In brief, all the data regarding the effects of conjugation derived from our study of Spathidium indicate a profound physiological stimulation of the metabolic activities of the cell which is expressed in reproduction.

As we know, endomixis is a periodic process coincident with the rhythms during the free life of Paramecium aurelia, P. caudatum and P. polycaryum.[13] In Paramecium calkinsi, endomixis is absent though rhythms occur; in Didinium and Uroleptus, according to Calkins, and in Spathidium, according to Moore, it occurs during encystment. In Paramecium calkinsi it obviously is not a necessary phenomenon, and the same conclusion has been drawn from data on Spathidium. But, when it does occur, has it a significant physiological effect?

In Paramecium aurelia and P. caudatum a higher division rate certainly follows endomixis, since recovery from a rhythm is coincident with endomixis. However, since

[13] Woodruff and Spencer, "Paramecium polycaryum, sp. nov.," Proc. Soc. Exp. Biol. & Med., 20, 1923.

in other Infusoria (*e.g.*, Paramecium calkinsi) recovery from rhythms regularly occurs in the entire absence of endomixis, it is impossible to definitely determine a causal relation between endomixis and recovery from rhythms in Paramecium aurelia and P. caudatum.

In pedigree cultures of Didinium, Calkins found that periodically all the animals encysted for purposes of reorganization, or endomixis, and accordingly he interpreted the process as possessing a survival value for the race. Mast in his studies of the same species did not find periodic encystment and stated it to be improbable that encystment is necessary for the continued existence of Didinium or that it has any appreciable effect on the death rate or fission rate.[14] The data from Uroleptus, however, certainly indicate, as Calkins says, that after encystation, during which endomixis takes place, the animals possess an initial vigor similar to that of an exconjugant. Furthermore, split conjugants, that is, animals separated or cut apart immediately after contact and therefore long before the definitive nuclear changes of conjugation have occurred, undergo an endomictic process which results in rejuvenescence exactly similar to that following conjugation. Apparently the mere contact of two animals initiates the nuclear changes for conjugation, and when conjugation is thwarted the cells meet the emergency by an endomictic process which typically only takes place in the species during encystment.

Weighing all the available facts the conclusion seems justified that when degeneration is in progress endomixis has a rejuvenating effect, and we thus reach the general result that *both* conjugation and endomixis have a similar effect under similar conditions—both meet the emergency of physiological degeneration *induced by environmental conditions* which are not ideal. The fact that Paramecium aurelia and P. caudatum are so widely distributed and are able to exist under such diverse surroundings can reasonably be interpreted as due to their having instituted endomixis in the free-living state. And furthermore, it is possible, we believe, to explain in part, at least, the fact that Jennings's remarkably intensive study of conjugation in Paramecium failed to reveal acceleration of cell division: endomixis, which was then unknown, tended to maintain his control lines at a high level. At all events, crucial experiments on the physiological effects of conjugation are only possible in forms in which endomoxis is not a complicating factor.

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[14] Calkins, "Didinium nasutum, I, The life history," *J. Exp. Zool.*, 19, 1915; Mast, "Conjugation and encystment in Didinium nasutum with special reference to their significance," *J. Exp. Zool.*, 23, 1917.

If the conclusion is secure that both endomixis and conjugation rejuvenate, it is clear that synkaryon formation is not the essential factor, since this does not occur during endomixis. The cytological evidence, which is solely from Paramecium, indicates that in endomixis the first two of the three 'pre-synkaryon' divisions occur, one of which during conjugation is usually interpreted as a reduction division, though recently Dehorne has claimed that it is the third division. Since, however, the nuclear changes in conjugation and endomixis are apparently fundamentally the same except for the gamete-forming division, endomixis in Paramecium offers strong physiological evidence against the interpretation of either of the first two divisions in conjugation as effecting chromosome reduction, because, if one of the divisions in endomixis is reductional, then there must be somehow, somewhere, a compensating mechanism, for which there is no cytological evidence and against which there is much physiological evidence.

Erdmann from studies on size variation after endomixis in Paramecium has found that variations arise, some of which are heritable.[15] These, however, apparently fall in the same category as the variations which Jennings and his colleagues have demonstrated in pure lines of various Rhizopods, and which are almost surely independent of segregation and recombination phenomena as understood in the metazoa. In short, the physiological effect, so far as rejuvenescence is concerned, common to both endomixis and conjugation must be sought in intracellular changes independent of those generally interpreted as following from genetic factors.

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[15] Erdmann, "Endomixis and size variations in pure bred lines of Paramecium aurelia." *Arch. f. Entw.-mech.*, 46, 1920.

The universal method of reproduction in the Protozoa, as, of course, in final analysis in all organisms, is cell division. But while in higher animals reproduction is usually associated with fertilization, in the Protozoa there is clearly presented the essential separateness of these processes since when fertilization occurs it merely punctuates a longer or shorter series of independent organisms which are the products of fission. Fertilization, however, is apparently neither of such universal nor frequent occurrence in the Protozoa as was formerly believed to be the case. Following the early observations of Dallinger and Drysdale on the life history of Monads and the discovery of fertilization in the Infusoria, it was confidently predicted that only further research was necessary to reveal it in all forms. And Schaudinn's beautifully conceived and executed studies on the life histories of a series of Protozoa from Endamoebae to malarial organisms seemed to justify the idea. But recent and intensive work on the Sarcodina and the Mastigophora has signally failed in many cases of the former and most cases of the latter to supply the critical evidence of synkaryon formation necessary to pronounce fertilization a fact, and it certainly must be left an open question whether it is not the exception, rather than the rule, in these classes. At all events, and this is the point of immediate interest, asexual reproduction is obviously adequate in the Protozoa, as it apparently is in certain, at least, of the Protophyta, to carry on the stream of life uninterrupted. It is yet premature to attempt to trace the evolution of sexuality in the Protozoa, but if one may be permitted to hazard a guess, it will prove to be of diphyletic origin and from some nuclear reorganization processes endomictic in nature.

Enough has been said to recall to mind the rapidly changing picture which the so-called dynamic aspect of the problem of fertilization has presented since the pioneer work on conjugation in the Protozoa. Its investigation has been fostered very largely by the belief that the key to the fertilization of higher forms must be sought in the conjugation of unicellular organisms; and the Infusoria, it is hoped, have not so far proved disappointing, when we bear in mind that they have had as long an evolutionary history as man and exhibit in their *multum in parvo* plan of structure a complexity as real as it is different.

THE FOOD OF GROUND SQUIRRELS

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So much of the territory of Western North America is the natural habitat of the ground squirrel and so greatly have they increased with the expansion of cultivated areas and so vast is their destruction to growing crops that a study of their food habits and an exact determination of the damage produced by them is of interest and importance. These animals are prominently represented by one genus, *Citellus,* of which there are many species, enjoying a wide range over the western half of the continent. The species upon which this study is based is the Columbian ground squirrel (*Citellus columbianus columbianus*) found in Eastern Washington, Eastern Oregon and Northern Idaho. Its life, though modified by unusual climatic conditions, is probably representative of the genus.

Observing these squirrels in the fields from day to day during the time of the harvest of their wild grasses and lupines, one is impressed with the aptness of the old generic term *Spermophilus,* which expressed so fittingly one of the chief characteristics of these little seed harvesters. It is a fascinating sight to see them, standing erect among the ripening vegetation, bear-like, gleaning their harvest with capable fingers and teeth. In season, purple lupines, the little rock clover (*Erodium*) and waving seed-ladened bunch grass (*Agropyron*) fill their fields with plenty. Here they toil through the hours of early morning and late afternoon sunshine, busily, in anticipation of the very urgent, present need of the all-important nourishment against the near approaching time of estivation. Not that they are filling, for themselves, their hidden treasure houses, but that they may store up the necessary fat as a body food reserve which must carry them past the long months of estivation and hibernation. With the return from hibernation they begin feeding upon green vegetation and bulbs of wild plants. This desire for green food increases during the growing season and is given over only when seeds of wild grasses begin to mature.

We have found that foods useful to them at some seasons are not at all so at others. For example, while green substances such as growing grain, alfalfa and wild plants as the balsam root (*Balsamorhiza*) are eagerly devoured while succulent and in the season of early spring, these same plants seem to be of no value to them at a season of maturing grains, even should their growth have been retarded or development delayed by late planting. In captivity, squirrels whose estivation and hibernation has been interrupted do not touch young succulent winter wheat in their yards in the autumn. At this time they are fond of sunflower seed or carrot which they eat in small quantities.

USE OF WATER

That this animal does not use water directly as drink may be attributed to many of the remarkable phenomena associated with its life history as the early estivation and correspondingly early return to activity from hibernation. That it prefers succulent vegetation to water accounts for the great damage done by the ground squirrel in growing grain, as is later shown in this paper.

Squirrels of this species were observed day after day, in the wild conditions, sometimes in a brookside pasture, but only once during the five or six years of this close observation were they seen to drink, and then there was some doubt about the observation. Many times they have been reported as "abundant in lowlands where they are attracted by water," but the fact is they were attracted there primarily by the green grass rather than by the water.

It was desired to see if this squirrel would drink water. Accordingly three females, in a large yard in which they were being observed, were given a glass dish a few days previous, so that they might become accustomed to the vessel. On the 28th of July, in the midst of a very dry time, they were given sunflower stocks and eagerly ate out the juicy hearts of these. On July 29, they were given nothing in addition to the natural food in the yard, which was very dry and parched. On July 30, the dish was filled with water. In a very short time one of the squirrels came out of its den and after looking about casually, went over to the dish and at once began to drink. She drank for a long time, lapping up the water quite as a cat would lap it, but with her tongue moving much more rapidly. After she had quenched her thirst, she went away and her place was shortly taken by the other two squirrels, who also drank as she had done. A like test was given young squirrels in another yard. One of them tasted the water, but they seemed to be expecting their food, and almost immediately began to eat carrot when it was given them. On July 31, the old squirrels were given the same dish with water renewed in it, placed in front of the hole as before, and about a foot away on the opposite side of the dish from the hole were placed two carrots, in such a way as to make it necessary for the squirrels to go past the water to reach them. Presently one came out. She sniffed the water, hesitated, then sniffed it again and went on to the carrot. The second one followed her example. The third one did not appear. That they do nicely without water is shown by the fact that they lived for two years in these yards without it.

FOOD MANIPULATION

The handling of food is very dextrously done by the ground squirrels. One was observed in the yards eating buds of balsam-root. It first nosed into a bunch of leaves, selected a half-grown bud, nipped it off and ate most of it. It then reached up and took a whole head in its mouth and with a sudden jerk detached it; then holding the head in its paws and standing quite erect so as to see well above the grass it began eating, devouring petals and all. When eating clover, they have been observed to seize the blades of the leaf in the mouth and the leaf stalk in the paws and pull until it breaks from the stem.

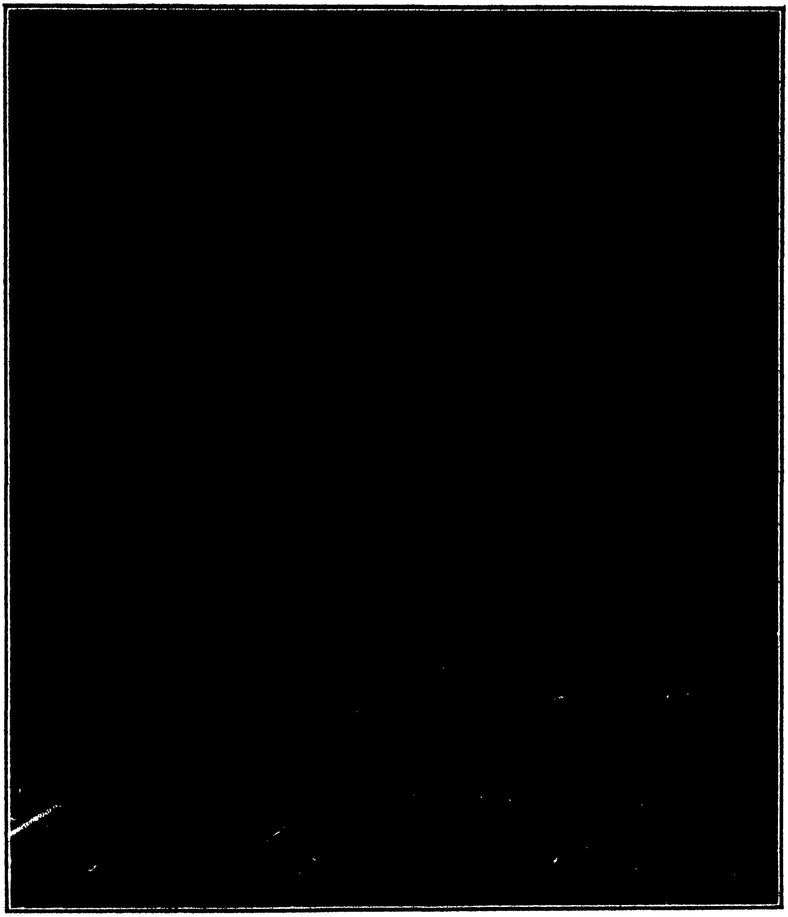

FIG. 1. Columbian ground squirrel (*Citellus columbianus columbianus*) in the midst of a wheat field. The heads of wheat scattered about have been deprived of the grain, yet are left intact.

Not infrequently the squirrel has been observed holding the stem of a clover head in one paw until it had finished devouring it, or even walking on three legs and carrying food in one paw. In gathering clover or other seed-bearing heads they grasp the stalks first and bend them down, often nipping them off and eating down the stalk until they get to the head.

They harvest the grain from the hulls of wheat and oats, leaving the heads so cleverly intact that one is deceived into the belief that they are still full of grain (Fig. 1). Their skill in securing parts of the stock is no less marked.

In the early season the squirrel feeds largely upon grass. It is especially fond of blue grass, and I have observed it in the yards seize a short blade of previously pastured blue grass between its incisors and pull straight up. Finally the blade would break from the crown and be worked back into the mouth by the process of chewing.

A very common squirrel-like attitude while feeding is sitting on the hind feet, tree-squirrel-like (*Sciurus*), manipulating the food with both front paws. Occasionally, when seated on their feet eating, they give the tail a flicker (only one flicker) apparently as an indication of pleasure.

This species of ground squirrel possesses the ability of extracting the tender stems of grain from the stock with a great deal of skill. It is done in this way: From about the 10th of June until the grain has formed it bites through the sheath on the side of the stock a few inches from the ground above a joint and very cleverly pulls out the tender, juicy end of the stock, leaving a tangle of wilted stems to mark the neighborhood of the den. So completely do they take the standing grain that scarcely a stock is left within a rod about and the grain in the vicinity of the holes forms a matted tangle of dead, shattered and crinkled stems adhering by shreds to stubble not yet dead (Fig. 2). From this tangle a second growth frequently springs and slowly develops in a new though unmaturing crop after the squirrels have gone into estivation. By July, when maturing grain finally renders the stocks of wheat or oats too dry for its purpose, attention is given to the ripening grain itself. At this time, when the grain is too high to reach, it cuts the stock off sharply or crinkles it and drops the head to the ground. It is not uncommon to find grain cut in this way two or three rods in from a fence row, as if pastured by cattle.

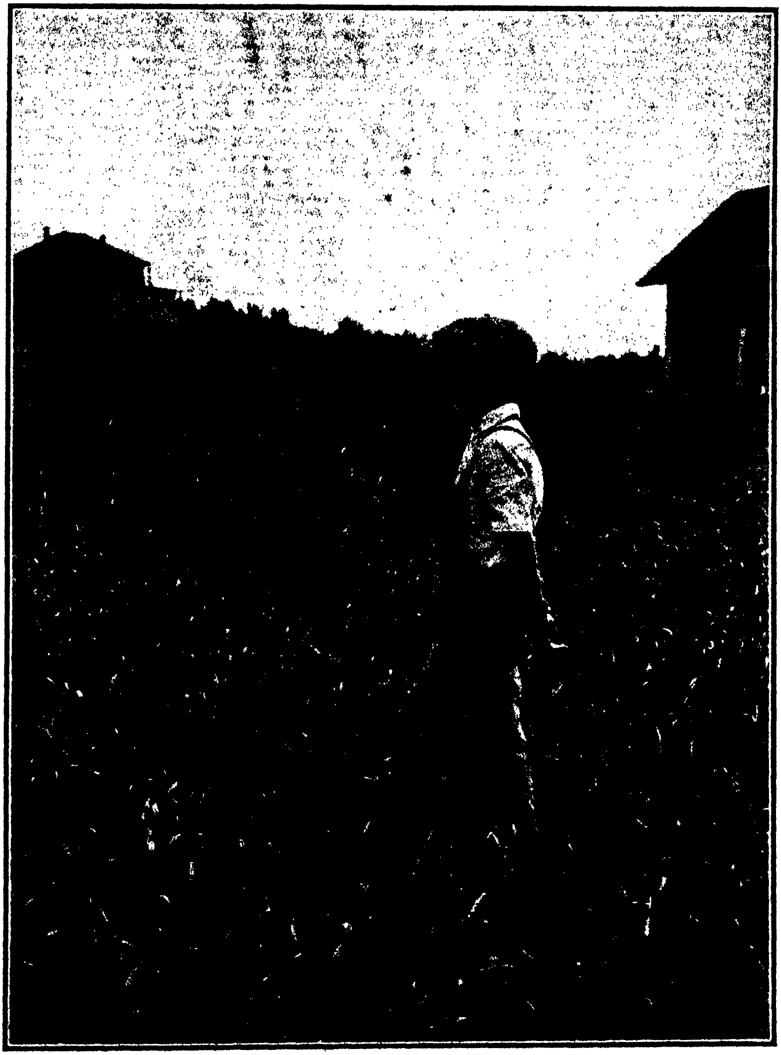

FIG. 2. Devastation in the squirrel-infested yard. The boy, four feet one inch tall, is standing where the stems have been cut since May 15. There is scarcely a head left for many feet around. Compare with Fig. 3. Picture taken July 1, 1919.

STORING OF FOOD

One might expect that these animals would store food for adverse conditions, but in no instance in the numerous investigations of the summer dens of this animal has there been found any trace or suggestion of stored food, although it is occasionally found in the nests of hibernating squirrels. Heads of wheat were rarely found and were almost entirely grainless. They had been used by the squirrel before going into estivation.

CULTIVATED VEGETATION

With the settling of the country, the squirrels were furnished with an abundance of plant food and at once adapted themselves to the use of such cereals as wheat, oats, barley and blue grass. They have a special liking for such legumes as clover and alfalfa. In the latter part of June and through July, 1910, it was noted that the squirrels were feeding upon bean plants, wheat, new potatoes, the juicy stems of corn stocks, the foliage of field peas, carrots and apple.

In the yards, the squirrels have shown a preference for carrots above other foods. Likewise, when they live near an orchard, they will go considerable distances for apples, especially in the spring when they secure the fruit which has passed the winter on the ground under the snow. These they carry to the mouth of their burrow before eating.

STOMACH ANALYSIS

A more exact determination of the food was secured by examining the stomachs. In preparation for the work, specimens were taken at various times of the day from June 10, until July 28, over a wide range about Pullman, Washington. The specimens were collected from such situations as stubble fields, wild oat patches, old pastures, orchards, wheat, oat, barley and pea fields, alfalfa fields, white clover pasture and unplowed fallow. It was a difficult matter to determine specifically the kinds of vegetable matter eaten, owing to the thorough mastication to which it is subjected. This is also true to some extent in the few insects and larvae found, for they appear to remove the skin, legs and mouth parts, eating chiefly the fat and muscle.

In general, the food of spring and early summer as shown by this study consisted of bulky, fibrous vegetable material such as would be found in grain leaves, grass and alfalfa, while later in the season the stomach contents were less bulky, but more concentrated in finely ground grain products. Animal and vegetable contents of the 43 stomachs examined at different times through the active season appeared in the following percentages:

100 per cent. contained vegetable matter.
86 per cent. contained vegetable matter entirely.
2 per cent. contained traces of mammal.
13.9 per cent. contained traces of insect remains.

The very low particles of insect remains sometimes found would lead one to believe that they were taken accidentally with other food, as in the case of lepidopterous scales found. Those insects clearly recognized in all these stomachs were three ants and two larvae of a carabid beetle (*Calosoma* sp.). One stomach contained some hairs, probably those of a mouse.

WILD VEGETATION

When squirrels come from hibernation, they may often be tracked in the snow to the whorls of yarrow leaves (*Achillea*) exposed by the sun, or they soon begin digging in the moist earth, probably securing bulbs of wild plants, such as Allium. There were signs of their having eaten buttercup blossoms, growing near their burrows, and later in the season wild oats were used by them. Strange to say, the bunch grass blades so common in their territory seemed of little use to them even when green and young and were resorted to as food only when other forms of vegetation were lacking in the yards. When driven by hunger, however, they would eat them.

The form of blue grass (*Poa* sp.) growing in the yards was eagerly sought. This they kept cropped down closely. They were fond of the buds of the wild roses and kept the leaves and blossoms nipped off some distance from the ground. Wild lettuce (*Lactuca*) was eagerly devoured by them. It was quite impossible to start clover of any sort where the squirrels were kept.

AMOUNT OF FOOD PER DAY

In conducting the experiments for this determination, the squirrels were fed as usual the day before. The night before the test the yards were carefully cleaned of all possible food and everything put in readiness for the beginning of the test early in the morning. To account for evaporation and the loss by weight from this source, checks of an equal amount of food were put in adjoining yards under exactly similar conditions and weighed each time the test was made. This, taken from the total loss of weight of the amount given the squirrel, will give the amount eaten. These tests were commenced early in the morning before the squirrels came out and were continued until late in the evening to make sure that they were through feeding.

The test of May 14 shows the following results:

1ST TEST, 6:30 A. M. TO 9:00 A. M.

Clover	4,067	grams
Clover check	4,067	"
Apple	57	"
Apple check	57	"
Clover eaten	9	grams
Apple eaten	9	"
Total	18	grams

Subsequent tests, using the same methods and material at 9:45 A. M. and 2 and 4 P. M. gave 8, 44, 21 and 44 grams, respectively, as the amount eaten, making a total for the day of 135 grams.

This test was carried on with a female, the mother of a family of young. At the beginning of the test she weighed 405 grams, the young gained nine grams, weighing 244 grams at the end of the test. The female consumed 33.3 per cent. of her weight.

In order that this test should be as natural as possible the clover was given as sod and was placed on a large glass and tin plate so that no dirt would be lost. Squirrels do not readily eat wilted grass or clover.

A similar test was made May 16, with the same squirrel, using wild white clover alone. On this date she weighed 412 grams and consumed 71 grams, or 17.2 per cent., of her weight of the clover from 6:40 A. M. to 7 P. M. Her four young were 21 days old and weighed 266 grams.

In a similar manner it was found that a young squirrel 4 months old, weighing 237 grams, consumed 106 grams of carrot on July 31; 71 grams on August 1; 100 grams on August 4, and 89 grams on August 5, being fed carrot exclusively during these tests.

THE PROBLEM OF SQUIRREL COST[1]

That the Columbian ground squirrel was very costly because of its injury to growing crops had long been recognized, but the actual amount of damage suffered had not been measured. An investigation was started, therefore, in October, 1918, for the purpose of ascertaining the damage done by squirrels to winter wheat. Positive measurable results were secured and are here reported.

THE METHOD OF STUDY

To further this investigation, a typical piece of fallow Palouse wheat land was selected and sown to a good variety of wheat. The weather being favorable, the wheat germinated promptly and soon covered the ground with a good stand.

Before winter, a part of this land was divided into sections 50 by 50 feet and a retaining fence three feet high and two feet in the ground was constructed about each plot to make it possible to hold the squirrels in absolute control. Within each of these plots was an old surface-obliterated squirrel den, such as is common in cultivated fields of the Palouse, even after the squirrels have been absent from them for many years. In these dens were many burrows too deep for the plow to crush. Once reached by the squirrel they were easily and quickly renovated and made into an inhabited dwelling, with plenty of wheat at hand. Immediately adjoining were check plots of similar size, slope and soil.

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[1] Popular Bulletin No. 118, Washington Experiment Station, Pullman.

The following spring all plots were cut back to 40 by 40 feet for an even stand, away from the fence. Wild squirrels were trapped, branded and otherwise carefully marked and recorded in preparation for the season's test and were placed in the enclosed plots as described below. The behavior of a representative male squirrel designated as Male No. 1 will be given in some detail; the observations made in regard to the others more briefly.

Male No. 1 was placed in an old den underlying the wheat plot in Yard 1, on March 22, at a time when the wild squirrels were beginning their work on the winter wheat in the fields. On the following day it began eating the succulent young leaves of the wheat. This it kept up steadily until the seventh of May, but the general detrimental effect upon the wheat was not noticeable other than the gradual thinning around the den. By May 9, however, a very marked, sudden and disastrous change had taken place. The wheat leaves were becoming less succulent and the squirrel, representing a species which is not dependent upon water for its liquid nourishment, had suddenly attacked the stems of the wheat plants and in the brief time elapsing had strewn the ground with wilted leaves and broken stems. In doing this it cut very skilfully into the side of the stem just above one of the lower joints and extracted a small bit of the tender juicy stem. Stem after stem had been cut to the ground and with each stem went the loss of one future wheat head. It is at this time that the wheat suffers its greatest devastation from the squirrel; for, where earlier a few bulky leaves and later an equally small number of concentrated heads would satisfy a squirrel's hunger, now it would take a large number of these very short tender stems to make a satisfying meal. From this date until May 27, the squirrel worked continuously upon the stems without variation, except that on May 23 it began cutting higher up on the stock in regions of more recent growth (Fig. 1).

On June 27, a change took place. The stems were now becoming woody in anticipation of approaching harvest. The squirrel abandoned its work on the stems and began feeding on the maturing grain. By this time the region about the den was well carpeted with a mat of dead leaves and stems.

In order to reach the heads of wheat the squirrel cut into the side of the stem up about the third joint, sometimes the fourth, usually just below the joint. In this way the stem was crinkled and the head dropped to the ground, and a matted tangle of wheat stems soon replaced stalwart waving stocks (Fig. 2). Many times the stem was completely cut off. In most cases the heads were then cut from the stem, close up to the grain, and carried to the mouth of a burrow and there the grain extracted and eaten. So skilfully was the grain separated from the head that the latter was left quite intact. By July 30, when the wheat was harvested, the squirrel had finished the depredations, accumulated sufficient fat for the long period of estivation and hibernation and was seen no more. The immediate vicinity of the den was strewn with empty wheat heads, and for nearly a rod around the ground was carpeted with bleached leaves and stems. From the area of the May and June stem cutting, now some green second growth heads struggled through, too green to mature and too short for the reaper.

The Result

On July 30, the several plots and their corresponding checks were harvested, the differences taken and the damage carefully calculated. It was found that during the 130 days of activity, from March 22 to July 30, the squirrel in Yard 1 had destroyed 44 pounds of wheat. Equal areas of 1,600 square feet were harvested, one squirrel infested and one free, showing in grain 89 pounds against 133 pounds, respectively. As a more striking comparison 500 square feet were harvested in the immediate vicinity of the den in Yard 3 (Fig. 2) and an equal area in the check plot for this yard (Fig. 3). The squirrel-infested area produced 4 pounds of grain against 41 pounds in the squirrel-free area (Fig. 4) and

Fig. 3. A normal stand of winter wheat in a check plot adjacent to the plot illustrated in Fig. 2. Photographed under exactly similar conditions.

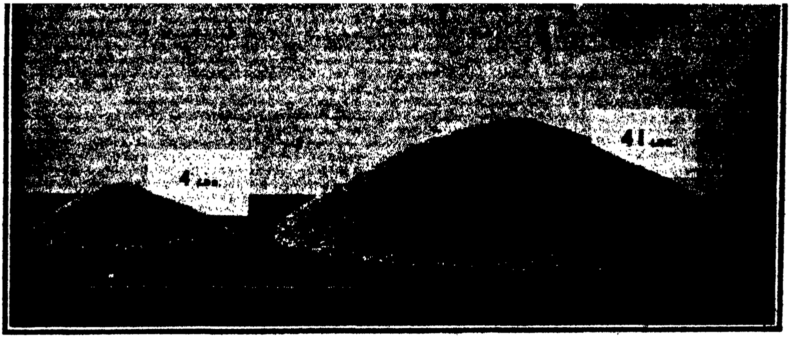

Fig. 4. Wheat secured from check plot and squirrel-infested plot. Forty-one pounds of wheat were secured from 500 square feet of the former as compared with four pounds of an equal area of the latter.

in straw, 1 sheaf against 9 (Fig. 5). This does not represent the entire damage done by the squirrel in Plot 3, as much was done at a greater distance from the den. A second test was made with a family of squirrels working on a 40 by 40 foot plot of wheat in Yard 2. Here the amount of wheat destroyed was 51 pounds.

In a third test, a male squirrel, Male No. 2, was placed in Yard 3 on May 15 to determine *when* the greatest

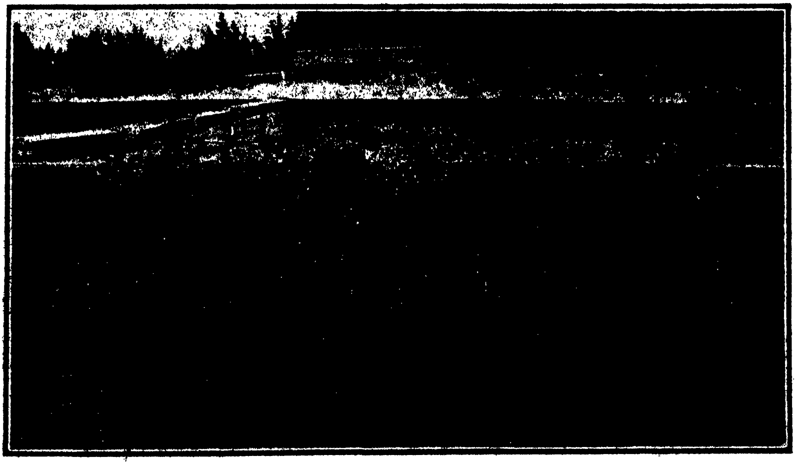

Fig. 5. Straw secured from check plot and squirrel-infested plot. Nine sheaves were harvested from the former, as compared with one from the latter. The retaining fences and plots are also shown.

damage was done to the crops. This squirrel was turned in this yard about a week after the squirrels in Yards 1 and 2 had begun the stem cutting. He remained there until time of harvest, July 30, and destroyed 56 pounds of wheat, showing that practically all the damage was done in the 76 days of stem-cutting and head destruction. The average destruction of winter wheat per squirrel per season in the experiments described therefore was one third of 44 plus 51 plus 56 pounds or 50 1-3.

GROUND SQUIRRELS AND PASTURE

Squirrels are very destructive to pasture and alfalfa, although their work is not noticeable to the casual observer as it is in grain. As already noted, one squirrel destroyed 71 grams of white clover in one day. At this rate, 385 squirrels would consume the pasture of one cow in a day or 96 squirrels would devour the pasture of one sheep in a day.

These tests were carried on under fairly normal conditions. It is true the squirrels were restricted to wheat and to whatever other plants were incidental to average wheat lands. It is also true that many squirrels live in exactly the same situations out in the center of large areas where they would have no other food.

NUMBER OF SQUIRRELS PER ACRE

During seven successive years, 7,000 squirrels were trapped on 416 acres of the college farm, or 2.4 squirrels per acre each year. A successful wheat raiser near Steptoe, Washington, trapped 1,200 squirrels on 200 acres in one year, or 6 squirrels per acre. This gives a fair estimate of the abundance of these animals.

In places where they are not controlled, they become very numerous and may destroy the entire crop. In an experiment on complete eradication conducted in one wild side-ravine pasture of 25 acres, adjoining wheat land, a careful estimate of 250 squirrels, or ten per acre, were destroyed by gas. Another area of old blue grass sodded bottom land showed an estimated concentration of about 25 squirrels to the acre.

THE EVOLUTION AND DISPERSAL OF THE FROGS

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OUR present conception of the natural groups of frogs and toads has been the outgrowth of many years of investigation by such scholars as Cope, Gadow and Boulenger. In 1882 Boulenger utilized in his classical "Catalogue" a modification of Cope's system (1864–1865). The structure of frogs soon came to be better known and as might have been expected, certain genera were found which did not fit well into Boulenger's groupings. From 1882 until the present day herpetologists have continued to add to our growing information, but not until 1916 was any radical change suggested which met with general approbation. Various authors contented themselves with stating that Boulenger's system was not expressive of genetic relationships, and some such as Gadow suggested minor changes. Boulenger in his last general work (1910) saved the trunk of his old tree by merely lopping off some of its most shattered branches. Recently I have had the occasion to bring together the data which have been accumulating during all these years and have tried to arrange this material in such a way that it would accord with the known facts of anatomy, paleontology and zoogeography (Noble 1922). Before this could be accomplished some preliminary papers by Metcalf (1920, 1921, 1921a) appeared, claiming that the origin and dispersal of the families of Salientia may be determined by a study of their opalinid parasites. These parasites Metcalf plainly tells us are in general not specific, one species frequently occurring in many different genera of frogs, in different families and even in salamanders and fish. The fact that the same species of opalinid was found in two frogs did not show that these frogs were related, for Metcalf tells us that reinfections of the parasite have occurred many times. I failed to see that Metcalf's data had any bearing on the past migration routes of the frogs and toads and so stated in my earlier paper (1922).

Since this time Metcalf (1923) has published his data in full. This detailed account confirms my earlier conclusions, and there would be no occasion for discussing the subject further were it not for the fact that Metcalf (1923a) has reiterated his claim in one of the recent numbers of this journal.

The evolution of the various groups of frogs and toads has been determined chiefly by a detailed study of their osteology, but all anatomical data, especially the myology, have been considered. The fossil evidence is much too scanty to be of value, but as there has been found a general agreement between the osteological, myological, ontogenetic and zoogeographic data there can be little doubt that the evolution of the group has been sketched correctly as to its main features. Metcalf, however, finds it convenient to fall back on some of the text-books which are admitted to be inadequate by all students of the group. In short, many of Metcalf's families of Salientia are known to be artificial, but this has not prevented him from making numerous broad generalizations in regard to their origin and dispersal.

The most primitive of living or fossil frogs are the *Discoglossidae,* and of these *Liopelma* and *Ascaphus* stand nearest to the labyrinthodont ancestors of the order. These two genera, widely separated geographically, agree among themselves in such fundamental features as the retention of a single coracoscapula (in addition to the "coracoid"), without acromion, the presence of a large pubis ("prepubis") and the presence of 9 presacral vertebrae which are not opisthocoelous—as usually stated—but really amphicoelous, for the intervertebral cartilages ("centra") never ossify nor do they more readily attach themselves to the vertebra immediately before than to the one behind. *Ascaphus* and *Liopelma* are unique among frogs in retaining two tail-wagging muscles (caudalifemoralis and caudalipubois chiotibialis), although neither possesses a tail. Further, *Liopelma* retains 3 pairs of cartilages lying in the inscriptio tendinea of the rectus abdominis, which because of their enormous size (nearly as large as the sternum), may very likely prove to be remnants of the ventral armature of labyrinthodonts and the gastralia of *Sphenodon.* From these primitive discoglossids the other genera apparently arose by the development of true opisthocoelous vertebrae and by the modification of pectoral and pelvic girdles. Other changes may be traced in the myology and especially in the urinogenital system. Although these modifications within the discoglossids are the subject of a study still unpublished, it may be mentioned here that *Ascaphus* retains as evidence of its lowly position both post cardinals, exhibits no fusion of Müllerian and Wolffian ducts in the male, and has a kidney which extends the entire length of the coelom. From some of the higher, purely aquatic discoglossids the *Pipidae* (Aglossa) have developed merely by an accentuation of those changes which have occurred as the extreme modifications of other aquatic frogs. A detailed statement of these changes have been given elsewhere (Noble 1922). The higher groups of frogs and toads have evolved from discoglossid or pro-discoglossid ancestors in somewhat divergent lines. The *Pelobatidae* form a natural group of primarily terrestrial or fossorial forms. The brevicipitids and ranids form a second natural group, the hylids and bufonids a third. In each of these three stocks of higher frogs various lines of descent may be traced. Certain changes have occurred within these lines which parallel similar modifications in other lines. Of the more conspicuous changes may be mentioned the reduction in the number of vertebrae, the fusion of the two halves of the pectoral girdle in the midline and the loss of the maxillary teeth. These lines of descent are not merely theoretical—drawn on paper by systematists—they are natural lines as shown by the sum total of external and internal characters as well as by the life history and distribution of the groups. The picture is complete for a large series of lines, but is still fragmentary for others.

When these lines of descent were grouped according to their natural affinities the question arose, Did the present distribution of either the larger or smaller categories demand the existence of former transoceanic land bridges to account for their distribution? In every family it was found possible to assume a holarctic dispersal, except for one recently evolved group, the brachycephalids, which still reside in their ancestral home, the neotropics.

Metcalf believes that his opalinid data give an important clue as to the routes of migration followed by the Salientia. Let us, then, examine his evidence in more detail. Passing over the discoglossids which Metcalf agrees arose in the holarctic regions we come to their relatives the pipids and find that unfortunately the American representatives of the family are not known to be infected by opalinids, and hence Metcalf's conclusions in regard to this family are pure assumptions. Turning to the pelobatids, Metcalf (1923, p. 361) tells us that "the opalinid parasites of this family throw little light upon the problem of its geographical distribution." And what information does he give us as to the higher families? The "Bufonidae are an Equatorian family" he writes (1923, p. 364), "dating back to the Triassic period." But Metcalf has studied the opalinids of only two genera! One of these, *Engystomops,* is not a "bufonid" at all, but a toothless derivative of the "leptodactylids." *Paludicola,* with which it agrees not only in osteological detail (witness the peculiar sternum, common to both), but also in regard to the essential features of its specialized life history (the eggs develop in a foamy mass of spawn in contact with the water). The other genus, *Bufo,* Metcalf (1923a, p. 403) admits "adopts any opalinids present in its habitat." Metcalf has not been fortunate in securing infections from any critical genera or families. He had before him not a single opalinid from any "dyscophid" (toothed brevicipitids), the ancestral stock from which all "gastrophrynids" were derived. Still, we should not discuss the latter family, either, for Metcalf (1923, p. 369) tells us that "the Opalinid parasites of the *Gastrophrynidae* do not throw much, if any, light upon the problem of their distribution." In short, Metcalf's data can be applied to only three families: the ranids, the toothed bufonids (leptodactylids) and the hylids. In regard to the first it is universally recognized that the oriental and African ranids are allied, and that *Rana* must have had a northern center of dispersal, but there is no evidence "that *Raninae* have lived in northern South America apparently since early Cretaceous times." These so-called "*Raninae*" are close relatives of the toothed bufonids. And they agree with South American bufonids not only in the critical features of their osteology and myology, but also in their opalinid parasites. They differ from bufonids in their solid pectoral girdle, and are therefore classified as a distinct family, the *Brachycephalidae.* Some brachycephalids still retain a partly arciferal girdle as definite evidence of their bufonid ancestry.

In regard to the "leptodactylids," Metcalf has nothing to add, save that certain opalinids and these frogs happen to be found in the southern hemisphere. Now the phylogeny of many genera of leptodactylids has been worked out in detail. It has been found that the end forms were almost invariably without maxillary teeth. Wherever in the world toothless "leptodactylids" (bufonids) occur it may be readily assumed that they have arisen from toothed forms. In certain regions, as in India, the toothed forms no longer exist, but the toothless genera are there as evidence that toothed forms either existed in this region or an adjoining one. Toothless bufonids can not be separated as a family from the "leptodactylids;" we must necessarily widen our conception of the family *Leptodactylidae*. When this is done it is found that the "leptodactylids," or, as we now call them, bufonids, have a world-wide range, and lend no support to the theses expressed by Metcalf.

We come at last to the one remaining family, *Hylidae*. The occurrence of *H. wachei* in Africa makes it difficult to assume a southern origin for the group. But Metcalf is attracted by the present hiatus of range in the Indo-Oriental region. He concludes (1923a, p. 394) "It seems wholly unlikely that Hylids, whether of Nearctic or of Palearctic origin, could have entered Australasia from the north without leaving Euro-Asia and Malaysia species, showing affinity to present Australasian tree frogs." Would Metcalf then have us believe that extinctions rarely occur? In any group where the fossil material is well known, as in the mammals, we have abundant evidence of such extinctions. Fossil Salientia are rare, especially well-preserved material. Recently it has been my good fortune to have placed in my hands for description a beautifully preserved pelobatid from the Oligocene of Mongolia. Here is a case of where we have definite proof of the former existence of a family in an area where it does not now reside.

It must, nevertheless, be admitted that the distribution of the *Hylidae* presents the weakest point in the evidence which I have brought together. And although Metcalf has added nothing to prove or disprove the theses submitted, his theories have recently received support from an unsuspected quarter in the able herpetologist and zoogeographer, John Hewitt (1922). While admitting the classification brought together by me to be a natural one, Hewitt would prefer to assume that the radiation into Asia, Africa and South America came, not from the north along land masses existing to-day, but from the south on three *theoretical* land masses existing in Mesozoic times. Antarctica, to be sure, enjoyed a temperate climate in Jurassic times, but proof that this land mass was connected with either South America, Africa or

Australia is still lacking. We have the fossil evidence that the mammals have migrated along existing land masses all over the world in Tertiary times. Surely the Salientia did not remain stationary during these 60 millions of years! Witness the flood of northern frogs (*Rana, Bufo, Gastrophryne,* etc.) which have entered South America since the Panama connection. The story of these wanderings is by no means complete as to detail, but all evidence points clearly to the fact that the migration routes lay along existing continental masses. Perhaps we can not say that the lowly toad accompanied the camels or marsupials in their wanderings, but at least they trudged along the same roads.

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PHYLOGENY IN THE ORIBATOIDEA

ARTHUR PAUL JACOT

IN an order of animals which are outstandingly predatory or parasitic, as, for instance, the Ixodoidea, which are parasitic chiefly on vertebrates, the Boellidae, which are predaceous on tiny animals, the Cheyletidae, which are both predaceous and parasitic, the Tetranychidae, which are chiefly suckers of plant juice, the Erythraeidae, which are chiefly suckers of animal juice, the Trombidiidae, which are parasitic as larvae and predaceous as adults, the Gamasidae, Sarcoptidae and others, we find one family which is distinctly herbivorous and non-injurious or saprophytic, filling a similar though far less conspicuous place in nature as do the ungulates and rodents among the mammals. They browse on vegetation or feed on decaying plant or (much more rarely) on decaying animal matter. In a group, the Acarina, which is characteristically positive, aggressive and well supplied with organs of attack, we find this one group, the Oribatoidea, which is negative, humble and devoid of weapons of offense.

Since in nature it is necessary for inoffensive animals, in order to maintain themselves on the surface of the earth, to be protected from the predaceous, it is interesting and instructive to consider how this isolation group is protected.

Although one of the chief characteristics of the Arthropoda is an external skeleton which acts as a coat of mail on the exterior of the body, the Acarina and Araneae have this dermal armature far less developed than is the rule in the majority of the other orders. An analysis of the various families and superfamilies of the Acarina for the purpose of comparing the amount of chitinization gives the following results:

Demodecoidea—very thinly chitinized.
Eupodoidea, Sarcoptoidea, Trombidoidea—soft and delicate.
Hydrachnidae—usually soft, sometimes with dorsal shields, more seldom uniformly chitinized though not strongly.
Halacaridae—with coriaceous plates or shields or entirely leathery.
Ixodoidea—fairly well chitinized, skin coriaceous to leathery.
Parasitoidea—with coriaceous plates and shields.
Oribatoidea—body quite coriaceous to highly chitinized.

The Oribatoidea, therefore, stand out from among their phylogenic relatives by reason of the extreme amount of chitinization.

A more detailed study of the group will, however, show that all the species are not so highly chitinized. Species, to be more specific, belonging to the genera *Hypochthonius, Nothrus, Neoliodes* and their related genera are quite as poorly chitinized as are species of other poorly chitinized superfamilies. In the genera *Scutovertex, Cepheus, Hermannia, Tegeocranus* (using these terms as did Michael) and their related genera one finds species much more fully chitinized but with the general appearance of the *Nothrus* group, while in the genera of the Eremaeinae, Oribatinae, Pterogasteridae and Ptyctima we find highly chitinized, smooth to glossy species, which are the climax forms for this one protective character. As a smooth, glossy sphere is more difficult to cut into than a roughened, angular form, the genera having reached this stage may be considered more advanced than the others—other things being equal.

A survey of the many forms of non-predaceous animals for the purpose of determining their various means of protection from enemies, besides that of exterior armor, will recall flight, development of special structures of rebuttal (as horns), leaping and retractility of soft parts within or behind hard parts or areas as in the foraminifera, bryozoa, brachiopoda, mollusca, barnacles, cladocera, turtles and others. Among the Oribatoidea three of these forms have been employed with apparent success.

Species of the subfamily Eremaeinae have recourse to flight and consequently have developed strong, fairly long legs (compared with the Hoploderma, Hypochthonii, Nothrii, etc.), each segment being broad, bulby, heavily muscled and articulated so as to give each a maximum of flexibility. In the Oribatinae the legs have become very much over-developed in relative length of each article so that the legs are insufficiently muscled to warrant rapid action. As a result, in animals which seem to be built for great speed, one finds slow, sluggish progress, much as in the herons, storks, pycnogonida, phalangoids, etc. In these forms the body is medium to small in size and not specially protected except by the chitinization. These species are developing into legs (carried to extreme in the Pycnogonida) for no good reason except that they are mutating in that direction. This group, then, is a climax group, marked by its highly chitinized exoskeleton and the extreme development of leg segments, either in girth or length, in comparison with the size of the body.

Species of the subfamily Zetorchestinae have developed the ability to leap when disturbed, by the development of the proximal segment of the hind leg, much as has happened in several groups of Arthropoda. They also may be considered a climax group in one of the major groups.

The third method of protection that is used by species of Oribatoidea is that of retraction of vulnerable parts within or behind hard parts. This has been accomplished by two clearly defined and separate methods. In the Ptyctima the upper posterior edge of the head (cephalothorax) is hinged to the upper anterior edge of the abdomen so that it may be bent downward against the antero-ventral part of the abdomen. Before this is done, however, the legs are drawn into the abdomino-cephalothoracic cavity. Thus the animal presents a hard, oval or flattened oval to its enemy. This, then, marks a climax development as the protection is complete. It might be here added that the subfamily Tritiinae, which I have discussed elsewhere, is more highly developed than the Hoplodermatinae (Phthiracarinae) by virtue of the fusion of the ano-genital plate to form two long and very narrow plates, and by the extreme compression of the abdomen and consequent narrowing of the venter or ano-genital area. In the Aptyctima the converse, as far as relation of cephalothorax and abdomen are concerned, is found. Protection to vulnerable parts is insured by special leaf-like or plate-like growths or expansions of the chitinous body covering behind or under which the vulnerable parts may be retracted. Of these expansions there are two distinct types. One type, called the pteromorphae, consists of an expansion from each side of the abdomen; the other type, the lamellae and tectopedia, consists of paired expansions springing from the cephalothorax. The presence of the former comprises the family Pterogasteridae, the absence of pteromorphae characterizes species of the other family, the Apterogasteridae.

The Apterogasteridae have been divided into the following subfamilies chiefly on the basis of development of legs. In the Camisiinae the legs are composed of short, squat segments with poor flexibility of motion, thus being much more fitted for exerting great pushing force than for flight. The body is poorly chitinized, rough and dull. Thus they are protected from attack only by their unattractive and inconspicuous appearance when on their habitudinal substratum. The lamellae and tectopedia, which would otherwise give them good service, are absent or insufficiently developed to afford any real protection. Thus, this is the least specialized or most primitive group of the family and should, therefore, be placed first in a linear arrangement. It is from this group that the runners and jumpers have been developed. In the Eremaeinae, as above mentioned, the legs are specialized for running. Their lamellae and tectopedia are of no efficiency and can only be considered ornamental or abortive structures. The body is usually smooth and shining. In the Oribatinae the legs have become very much overdeveloped and the lamellae are still less developed. They thus form the climax of this line of specialization. The Zetorchestinae, as above discussed, would also fall into this family, having specialized in jumping instead of running. The Serrariinae, consisting of species which have specialized in food habits because of the peculiar development of the mandibles, form the fifth and last subfamily. Nowhere in this family do we find such extreme development of lamellae and tectopedia as in the next. One would not expect to find rapid runners with large and cumbersome lamellae except as one of those overdevelopments which nature occasionally indulges in. The subfamily might be further subdivided into tribes as outlined by Ewing (1917, June), but it would add nothing to the comprehension of the group to multiply the number of subfamilies.

Turning now to the Pterogasteridae, one is bewildered by the large number of genera and the arbitrary way in which they follow one another in systematic papers. A study of the group, however, reveals definite lines of specialization. Aside from the Pelopinae which have specialized (though not as far as the Serrariinae) in food habits and are consequently discriminated by their diminutive mandibles (or, conversely, have specialized in food habits *because of* the mutation of the mandibles), the genera of this family are marked by the greater or lesser development of their pteromorphae, lamellae and tectopedia.

Taking up first the pteromorphae, we find they have become specialized along two distinct lines. In the genera related to the genus *Galumna,* these structures have become very large, passing well beyond the anterior edge of the abdomen but, more important, they have become distinctive structures hinged to the abdomen and capable of being raised or depressed. They thus form enormous lateral wings or covers which, when the legs are withdrawn into the cupboards on each side of the abdomen, close down over the edges of the cupboard and completely enclose the legs and pseudostigmatic organs. Thus the animal is as well protected (except for the mouth) as are the species of Hoplodermatidae. Furthermore, these species are smooth and shining and the lamellae and tectopedia are very small to practically lacking as such. Thus, the specialization is complete and this as a climax group may be placed in the subfamily:

Galumninae subfam. nov. Type genus: *Galumna* Heyden, 1826. Pterogasterinae having the pteromorphae very large, extending downward to cover part of the ventral plate and forward to cover part of the cephalothorax, legs received in cupboards in the sides of the abdomen and entirely enclosed by the pteromorphae, pteromorphae *hinged* to sides of abdomen, lamellae and tectopedia quite small to lacking as such.

As thus delineated this subfamily would include the genera *Galumnella* Berl., 1916, *Neoribates* Berl., 1914.

Another line of development of the pteromorphae is found in the group of genera which are grouped about the genus *Oripoda.* In these the pteromorphae are also enlarged beyond the anterior edge of the abdomen, but they are not hinged to the abdomen. They are, on the other hand, united about the base of the abdomen and overhang the cephalothorax. The lamellae may or may not be developed (depending on the amount of development of the pteromorphal bridge) and a translamella may or may not be present. This line of special development may be known as subfamily:

Oripodinae subfam. nov. Type genus: *Oripoda* Banks, 1904 (Sept.). Pterogasterinae in which the pteromorphae are very much developed anteriorly and joined to each other along the anterior edge of the abdomen forming more or less of a bridge over the cephalothorax. This bridge may or may not be more or less fused to the cephalothorax. The following genera would therefore be placed therein: *Achipteria* Berlese, 1885, *Gymnobates* Banks, 1902, *Tenuiala* Ewing, 1913, *Jugatala* Ewing, 1913 (of which *Neogymnobates* seems to be a synonym). In none of these genera do the pteromorphae seem to be particularly attached to the cephalothorax. If such should, however, be the case the character would be of generic value only, as it would be secondary to the development of the pteromorphae *over* the cephalothorax.

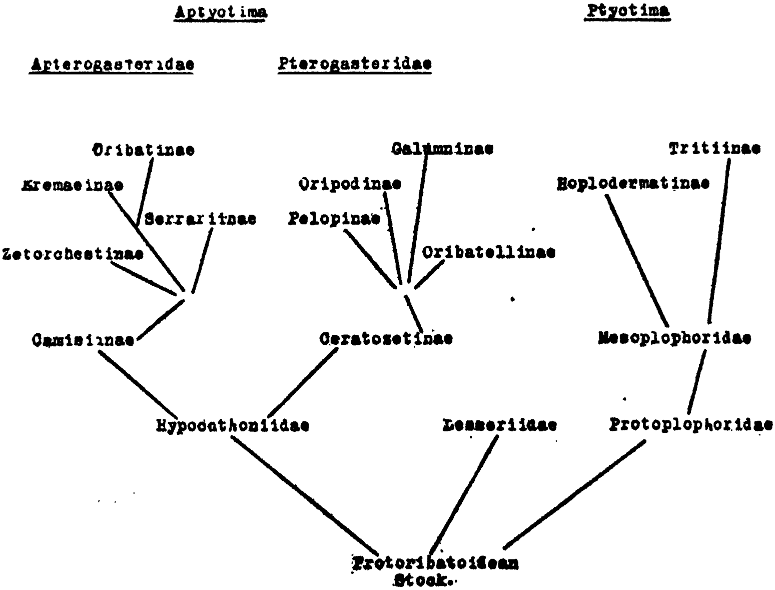
The lamellae have also developed along two distinct lines, one in which the lamellae are attached to the cephalothorax along their posterior edge only and one in which they are attached to the cephalothorax along their mediolateral edge as well as their posterior edge. The first group is typified by the genus *Oribatella,* and may be known as subfamily:

Oribatellinae subfam. nov. Type genus: *Oribatella* Banks, 1895. Pterogasteridae in which the pteromorphae are not developed beyond the anterior edge of the abdomen, but in which the lamellae are attached to the cephalothorax along their posterior edge only and are usually of relatively large size (*i.e.*, covering over a fairly large part of the cephalothorax). It would thus include, among others, the following genera: *Lepidozetes* Berl, 1910 (= *Tegoribates* Ew., 1917), *Joelia* Oudms., 1906.

The remaining genera, characterized by having the pteromorphae small, at least not extending anteriorly beyond anterior edge of abdomen, and by having the lamellae more or less attached by their inner lateral edges to the cephalothorax, and thus presenting no special line of development, may be grouped in the subfamily:

Ceratozetinae subfam. nov. Type genus *Ceratozetes* Berl., 1908. Pterogasteridae as characterized in the above paragraph. This group might further be divided into two tribes based on presence or absence of translamella. As this character is not clean cut, and until it can be shown to be a definite line of specialization by finding species in which the translamella is very much developed or overdeveloped, there would be no point in making a separate subfamily of it.

These groups and their relationships may be more easily visualized by reference to the following diagram:



This relationship, if arranged in linear form, would be: Hypochthonidae; Lohmanii, Nothrii, Tegeocranii in the Camisiinae, Serrariinae, Zetorchestinae, Eremaeinae, Oribatinae in the Apterogasteridae, Ceratozetinae, Oribatellinae, Galumninae, Oripodinae, Pelopinae in the Pterogasteridae; Lesseriidae, Protoplophoridae, Mesoplophoridae, Hoplodermatidae and Tritiinae in the Ptyctima, rather than the arbitrary order introduced by writers early in the nineteenth century. Such an arrangement would present the elements of the group in their natural sequence or order of development and would therefore be a more logical arrangement.

SHORTER ARTICLES AND DISCUSSION

THE HARE-RABBIT, A STUDY IN EVOLUTION BY HYBRIDIZATION

ONE view concerning the method of evolution, which has many supporters at the present time, is that hybridization is one of evolution's principal agencies. This view is usually advocated as an alternative to the view that variations, which form the material for progressive evolution, arise chiefly within the species, by spontaneous variation.

The latter view was held by Darwin, and as an illustration of it he cited the varieties of domestic pigeons, all as he thought derived from the same wild species, the rock pigeon, *Columba livia.* Darwin's interpretation has been challenged in recent years by Ghiji, who has shown that domestic pigeons can be crossed with one or two wild species other than *Columba livia,* resulting in the production of fertile hybrids. This result does not, of course, show that historically the production of domestic varieties of pigeons was actually accomplished by that method, since it may after all have occurred by mutation within the single species, *Columba livia,* with which all varieties of domestic pigeons are fertile. Nevertheless, it establishes the possibility that hybridization *may* have occurred and acted as an accelerator of the evolution of domestic varieties.

Another domestic animal, which has varied perhaps as extensively as the pigeon, and which has figured largely in discussions and experimental studies of evolution is the rabbit. As in the case of the pigeon, the chief, if not exclusive, ancestral species still exists wild, and domestic varieties are fully fertile with it. This is the wild rabbit of Europe, *Oryctolagus cuniculus.*

Domestic breeds of rabbits differ greatly in size, color, ear-length and proportions of the body. The smaller breeds are of about the size of wild rabbits, but other breeds are four or five times as large. This latter fact has led many to suppose that the European hare, *Lepus timidus,* which is much larger than the wild rabbit but similar in appearance, has crossed with rabbits to produce these large breeds, and that genetic heterogeneity resulting from the cross has probably produced further variability among domestic breeds.

The question of possible hybridity between the hare and rabbit has been hotly debated in Europe for half a century both by practical rabbit breeders and by scientists. Supposedly conclusive experimental evidence has been brought forward more than once that the two species do cross, but has always turned out in the end to be *not* conclusive. The "Belgian hare" purports in its name to be of hare origin, but it turns out to be so only in name, for (1) it burrows like other rabbits, (2) has young born naked and with closed eyes, and (3) is fertile with all other rabbit varieties. On the other hand, hares (1) do not burrow, (2) have young born furred out and with eyes open and (3) admittedly rarely, if ever, cross with rabbits. The burden of proof rests with those who think hares *can* cross with rabbits.

From a personal communication, I gather that Professor Lotsy, who regards hybridization as the chief if not exclusive method of evolution, is one of those who believe that in recent years a skilled rabbit breeder in Holland has actually made under controlled conditions a cross between a male hare and a female rabbit, resulting in the production of fertile hybrids. These admittedly resemble rabbits and produce young hairless and with closed eyes. Yet Lotsy believes them to be genuine hybrids. But conclusive evidence in support of this view has so far as I know not yet been published.

In this uncertain state of affairs, it is gratifying to learn of a thoroughly critical experimental investigation of the question recently made by two Japanese zoologists, the results of which appear to be conclusive. I wish to call attention to it, not only because of its intrinsic merits, but especially because being published in a Japanese journal in the Japanese language (with only a summary in German), it is likely to be overlooked and its bearing on general questions is likely to go unnoticed. The authors are J. Yamane and T. Egashira. The article is found in *Dobitsugaku Zasshi*, a monthly magazine of zoology published by the Zoological Society of Japan, Vol. 36, No. 430, 1924. It deals with experiments, attempted both by natural and artificial means, in crossing the domestic rabbit with the Japanese subspecies of the European hare (*Lepus timidus ainu* Barr.-Ham.). The results were as follows:

(1) Attempts at breeding hares in captivity were fruitless on account of the extreme wildness of the animals, in agreement with all similar attempts previously made in Europe.

(2) Attempts at crossing hares and rabbits reciprocally by natural means were without success. The hare would in no case consent to mate with a rabbit.

(3) A young female hare weighing 540 grams which had been recently captured was put in a pen with a male rabbit of like size, in the hope that the two might be reared together and thus become friendly, but without success. The hare refused to eat until removed again to a separate pen.

(4) An adult female hare was put in a large grassy outdoor pen with a male rabbit but was found next morning bitten to death by the rabbit.

(5) Attempts at artificial insemination of female rabbits resulted as follows. Sperm was taken from the epididymus of male rabbits and after dilution with a 3.3 per cent. solution of dextrose was injected into the uterus of the female. In 3 out of 36 cases pregnancy resulted. In another group of cases the sperm injection followed copulation with a male rabbit, rendered sterile by ligaturing of the *vasa deferentia.* In this group of cases the success was greater, pregnancy occurring in 15 out of 24 cases, or 62.5 per cent. of the trials. This result confirms the earlier finding of British zoölogists that in the rabbit ovulation is not spontaneous but is largely dependent upon copulation.

Having now developed a technique satisfactory for successful artificial insemination of rabbits, the authors next substituted hare sperm for rabbit sperm in their experiments, confident that if rabbit eggs can be fertilized with hare sperm, they would be able to produce hybrids.

Sperm in which lively movement could be observed under the microscope was taken from the vasa deferentia of hares, diluted with dextrose solution, and then injected, as in the experiments previously described, into the uterus of a female rabbit which had just copulated with a sterile male rabbit. In 38 cases the result was entirely negative.

In another set of experiments the procedure just described was followed and then from one to five hours later the female was allowed to copulate with a normal fertile male rabbit. In two out of five cases, pregnancy resulted and eight young were produced—all true rabbits, not hybrids.

The authors conclude that in their experience it is impossible to get hares to mate naturally with rabbits, and even if this were to occur under exceptional circumstances, no hybrid offspring would result because of the evident inability of the rabbit egg to be fertilized by hare sperm.

We may accordingly relegate the hare-rabbit to the limbo of zoological myths, along with the unicorn and the sea serpent.

Those who regard evolution as a consequence of hybridity alone will need to look elsewhere for proof.

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HAS PARTHENOGENESIS BEEN CONFUSED WITH HERMAPHRODITISM IN CUCURBITA?

In a recent paper by A. C. and A. L. Hagedoorn[1] experimental evidence is given that tends to show that parthenogenesis occurs in the cucurbita.

Since Lotsy's criticism in 1919 of a former paper by these authors we have been running check experiments, the results of which do not support the European workers.

Work was done on stimulation of stigmas in 1922. This consisted of cutting and crushing of tissues and placing foreign pollen on the stigmas. No fruit development occurred.

During the past season 301 female buds were tied and bagged as a double check against insect invasion. No fruits developed. As 57.9 per cent., 1,005 out of 1,733, of the blossoms pollinated matured and the fruits were harvested, it can be concluded that in the strains of *Cucurbita pepo* used parthenogenesis does not occur.

In a few open pollinated hermaphroditic fruits maturity occurred where the fruit stem was short.

Some of the figures in the Hagedoorns' paper show the characteristic receptacle cracks and navels of hermaphroditic fruits.

As these figures show individuals belonging to the genera *C. pepo* and *C. maxima* being crossed and fertile offspring resulting, the results seem to be directly opposite to those obtained by workers in this country.

If, however, they bagged a hermaphroditic fruit of *C. maxima* their results may be explained on the basis of being direct pure line descendants rather than descendants of a generic cross that has constantly been proven sterile. Figures 1 and 7 in their paper suggest such a condition.

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[1] Hagedoorn, A. C. and A. L., 1924, "Parthenogenesis in Cucurbita." *Zeit. Ind. Abst. u. Vererbungslehre*, pp. 186–213, figs. 1–8.

Russell, p. 1924, "Identification of the commonly cultivated species of cucurbita by means of seed characters." *Jour. Wash. Ac. Sci.*, pp. 265–269, figure 1.

Further work will be carried on here as a check on the results of workers on the cucurbita that have been published to date.

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EGG-LAYING IN TWO SPECIES OF PLANORBIS

FIVE individuals of *Planorbis corneus* L., and twenty-one of *P. trivolvis* Say,[1] were kept in a large balanced aquarium, except during the periods of experimentation described below, during the winter and spring of 1922–1923, and observations were made upon the number of eggs laid, and the periodicity of the process.

Among the *P. corneus* specimens egg-laying began on December 30 and continued to February 5. During this time 20 masses of eggs were deposited upon the glass walls of the aquarium and five upon the stems of water plants. No eggs were found elsewhere, and in all cases the eggs were laid at night. The number of eggs in the masses varied from 13 to 27, and the total number of eggs in all the masses was 456. As egg-laying continued the number of eggs per mass steadily decreased. The data give the following averages: 18.2 eggs per mass; 5 masses or 91.2 eggs per individual. Soon after discovery the eggs were isolated and proper precautions taken to allow the normal development of the embryos. Ninety-two per cent. of the eggs succeeded in hatching from the capsules, but after that stage the mortality from bacterial and fungal infection was so high that only 21 individuals survived through metamorphosis.

The 21 *P. trivolvis* specimens began egg-laying on April 12 and continued until April 23. A total of 872 eggs were laid; 32 masses on the glass walls of the aquarium and 3 on leaves of water plants. All of them were deposited during the night, and as egg-laying continued the number of eggs per mass decreased. The averages were as follows: 21.3 eggs per mass; 2 masses or 41.5 eggs per individual. No precautions were taken to allow these eggs to develop.

It appears therefore that *P. corneus* lays over twice as many masses and eggs per individual as does *P. trivolvis;* although the latter lays more eggs per mass than does *P. corneus.* It is further evident that both species deposit the majority of their eggs on the glass walls of the aquarium, that they both lay their eggs exclusively at night, and that as egg-laying continues the number of eggs per mass decreases. The number of eggs laid by other species of snails varies considerably. Baker[2] (p. 271) describes an individual of *Limnaea caperata* which deposited 5 masses between April 16 and 19, containing 45, 42, 42, 35 and 28, respectively, totaling 192 eggs, or an average of 38.2 per mass. In the case of Physa gyrina, Baker says (p. 314) that 15 specimens deposited 14 masses from April 23 to 24, three of these containing 130, 160 and 200 eggs per mass, or an average of 163.3. No indication is given of the chronology of laying of the three masses. It is further stated (p. 360) that *Valvato tricarinata* "lays from 10–30 eggs," with the inference that these figures represent the total number of eggs laid. Clapp[3] reports the number of eggs in masses from *Ancylus fuscus* as 7 to 8 at first, but that in the capsules deposited later the number decreases steadily down to 1. Anderson,[4] who studied the breeding habits of three species of Bullinus under controlled experimental conditions, gives the following data: Number of eggs per mass, 1 to 11; maximum number of eggs per individual, 19; total number of eggs, 138, deposited in 35 masses by 17 animals. His figures indicate that the number of eggs per mass did not increase as egg-laying continued, and that the length of the egg-laying period varied greatly among the different animals. Although he noted that copulation occurred during the daytime as well as at night, he does not say when the eggs were deposited.

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[1] The writer is indebted to Bryant Walker for identifying these species.

The fact that *P. corneus* and *P. trivolvis* laid their eggs only during the night raised the question as to whether or not the periodicity was controlled directly by the environmental conditions of temperature, illumination and carbon dioxide content of the water. During the night the temperature decreased five degrees or more, light was absent and the CO_2 content of the water increased, due to the cessation of photosynthesis in the water plants. The three factors, therefore, exhibited a daily rhythm of maximum and minimum. To determine any relations between this rhythm and egg-laying, 24 individuals were isolated in separate jars. Twelve were kept in continuous darkness, and 12 in continuous light, by using a 150-watt Mazda lamp, 30 cm. above the jars, and separated from them by a water heat screen. Records of temperature showed the same variations during the day and night as those of the large aquarium. At the end of 96 hours, after which 18 egg masses had been deposited, the experiment was discontinued. All of the eggs had been laid at night, 10 masses by the "dark" animals, and 8 by the "light" ones. In the second experiment all of the animals were kept in continuous light during the night and in darkness during the day for 96 hours during which time the temperature varied less than 2° C. Under these conditions 14 masses were deposited, all of them during the night, even though the normal environmental conditions of day and night had been reversed. Further tests were impossible because of the cessation of egg-laying.

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[2] Baker, F. C., 1902, The Mollusca of the Chicago area. Bull. no. 3 of the Natural History Survey, Chicago Acad. Sciences.

[3] Clapp, W. F., 1921, Eggs and young of the river limpet, Ancylus fuscus C. B. Adams. Occasional papers of the Boston Soc. Nat. Hist., vol. 5, p. 5.

[4] Anderson, C. W., 1923, Enquete et recherches sur la bilharziose en Tunisie. Arch. Institut Pasteur de Tunis., T. xii, no. 1. (For this reference and for quotations from it, the writer is indebted to Dr. W. J. Crozier.)

It is concluded that egg-laying in these species is controlled by a periodicity which must be regulated by internal processes, and not directly by the external environmental conditions of light, temperature and CO_2. The possibility of changing the rhythm by reversing the normal conditions over a long period of time preceding egg-laying is of course not precluded. Duirnal periodicity in the deposition of eggs in other animals is a common occurrence. For Gasteropoda, however, the references are few. Eales[5] (p. 71) says that for *Aplysia punctata*, the Sea Hare, "deposition usually takes place at night in the aquarium," and Clapp[3] reports (p. 5) that *Ancylus fuscus* laid all their eggs at night. This sort of periodicity then must belong to a different category from that found in Copepoda, whose daily depth migrations seems to depend directly upon the conditions of illumination, temperature and CO_2 content of the water,[6] and from that found in other animals due to similar environmental changes.

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[5] Eales, N. B., 1921, Aplysia. Liverpool Marine Biological Memoirs, no. 24. Liverpool.

[6] Loeb, J., 1905, Studies in general physiology, Pt. 1, p. 282. Chicago.

A HISTOLOGICAL METHOD FOR THE STUDY OF PIGMENTATION IN MAMMALIAN EPITHELIUM

THE object of the following note is to record a histological method for the determination of the location of pigment in the skin of rodents.

The material used in this work was epithelium from the tails of black mice. Some of these mice were uniformly black in color, while others had white spotting on their tail. Separate discussion of these two varieties is not necessary for the purpose of this paper.

According to many authors, pigment is situated directly beneath the epidermis. Bohm, Davidoff and Huber (page 404) say that pigment in the skin can best be studied in unstained sections. Guyer (page 49) gives methods for preparing skin for histological study.

Following Guyer's method in the case of pigmented skin proved inadequate, for although the cellular detail was good, there was no trace of pigment granules. An attempt was made to follow out Bohm, Davidoff and Huber's suggestions of using unstained preparations, but insufficient detail resulted, although there were visible pigmented areas.

After some experimentation, the following method proved to be successful and is recorded in the hope that it may be of service. The mouse was killed, a superficial incision was made around the tail, about three fourths of an inch from the tip. By gently twisting and pulling, the epithelial tissue came off as a sheath leaving behind the skeletal and connective tissue. The sheath was then split longitudinally, spread out flat and bound in position by fine silk thread to a flat piece of cork about one inch long by one half inch wide and one eighth of an inch thick. Thumb tacks stuck into the bottom of the cork served to keep it in an upright position immersed in the fixing solution.

The following method of fixing, sectioning and staining was found to be the most successful:

Fixation in Bouin's for three hours; three washings of 70 per cent. alcohol for 20 minutes each; 85 per cent. alcohol for 20 minutes; 95 per cent. alcohol for 20 minutes; absolute alcohol for 45 minutes; xylol for 30 minutes, and 45 degree melting point paraffin in an oven constant at 50 degrees, for one hour. Then the specimens were imbedded and sectioned at seven micra in thickness. They were stained in Delafield's hematoxylin for five, ten or fifteen seconds. Each of these gave excellent differentiation of pigment and cell structure. The five second slides were, on the whole, the best. Longer staining showed clearly that the pigment became either masked or destroyed.

It is not known how rapidly the sections will fade, but at three months after preparation, they are still entirely satisfactory for general study.

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THE LIFE CYCLE OF MACROSIPHUM SOLANIFOLII WITH SPECIAL REFERENCE TO THE GENETICS OF COLOR

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THE large aphid of the potato and the rose, *Macrosiphum solanifolii,* exists in two color varieties, green and pink, respectively. The precise relation of these varieties to each other has hitherto been unknown. Patch (1915) reared 500 progeny, all pink, from nine original pink parents, but in correspondence with other entomologists it is sometimes stated that the species "throws sometimes green, sometimes pink" individuals, as if there were a suspicion that one or both of the color types might produce both kinds of offspring. Certain facts related below could easily lead a casual observer to think that at least the green variety sometimes produces the pink, and it is not unlikely that the idea has been entertained that either variety may produce both. There is nowhere in the literature, so far as I can find, any information concerning the relations of the two varieties through sexual reproduction. The work described in this paper was undertaken to ascertain the facts on these points.

The species exists in eight different forms, four in the green variety and four in the pink. Thus, there are apterous parthenogenetic and alate parthenogenetic females, oviparous (sexual) females and males in each color variety.

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[1] Contribution from the Zoological Laboratory of the University of Michigan.

DESCRIPTION OF THE EIGHT FORMS

The more striking structural characters are the same in corresponding forms in both color varieties, and are given here for the guidance of those who may wish to study the species. The apterous parthenogenetic females have 4 to 6 sensoria on the third segment of each antenna, no sensoria on the hind tibiae and have no ocelli. The alate parthenogenetic females have three ocelli, 15 to 18 sensoria on the antennae and no sensoria on the hind tibiae. Oviparous females are wingless, have 4 to 8 sensoria on the antennae and hundreds of sensoria on the hind tibiae, which are swollen and brown. Males, which are winged, have 25 to 40 sensoria on the antennae and none on the hind tibiae.

The physical basis of the color in each of the eight forms is briefly described in the following paragraphs.

Pink Apterous Parthenogenetic Female: The pink color of this form is due to minute spherules of a pink or red color contained in the body fluid. The largest of these spherules are about 1 μ in diameter, and the smallest presumably below the limits of visibility. There are hundreds of thousands of them in a single aphid. Being in the body fluid, these spherules are crowded aside by the organs or definite tissues, or by larger bodies of other kinds contained in the body fluid. The color pattern is therefore somewhat variable in different individuals because it is determined in part by the distribution of the internal organs, including the passages filled with unborn young, in part also by inequalities in the distribution of the red spherules in spaces which they might occupy.

Very young embryos while still in the body of the mother do not possess any of the red spherules and are colorless; but the red substance appears before birth. A color pattern is usually more definite in the young than in the adult. There is, in these young insects, a colorless strip along the middle of the dorsum of the abdomen, due to the heart and principal vessel, and a segmental arrangement of the color on both sides of this strip. About seven blocks of red on each side of the middle line are separated from each other by colorless strips. The thorax and head of the young aphid are of a paler red color, due to fewer red spherules.

Pink Alate Parthenogenetic Female: The pink color is due to red spherules of the same sort as those in the apterous female. For the most part they are no larger than in the apterous female; but in some specimens a few larger globules, up to 2 or 3 μ in diameter and of a reddish orange color, have been found. These larger orange globules may be of a different substance from the smaller red ones.

The distribution of these red spherules differs from that in the apterous form in certain respects. Thus, there are few of them in the meso- and metathorax, owing to the great development of the muscles there, so that this region is yellowish. Also there is not infrequently a honeycomb arrangement of the red spherules in the dorsal region of the abdomen, which is due to the presence of larger globules of a colorless or faintly yellowish substance thought to resemble fat, in the body fluid, with the red spherules crowded between them and collected on their surfaces.

Pink Oviparous (*Sexual*) *Female:* The color of the pink oviparous female is due primarily to red spherules not different from those of the parthenogenetic female. These spherules are, however, usually distinctly more abundant, so that the oviparous females are more opaque. This opacity is observable frequently in the early nymphal stages, so that the sexual females can be recognized before the distinctive structural features appear. In addition to the red spherules there are often numerous globules of larger size, up to 5 or 6 μ, of a bright yellow color, which no doubt help to render the aphid more opaque than the parthenogenetic females, without modifying to any considerable extent the general red color.

The eggs of the pink oviparous female are bright green in the body and when first laid, but turn black later. Accumulation of eggs in the female's body displaces the red spherules and gives the whole insect at first a paler pink, then a greenish appearance.

Pink Male: The basis of color in the male is more variable than in any of the females. The body fluid contains large numbers of globules of varying size and color in different individuals. In one specimen they were found to range from the smallest visible size up to 13 μ; in another from 1 to 20 μ; in a third 4 to 10 μ; while in another none were larger than 3.5 μ. In one individual all globules up to 4 μ in diameter were colorless or of a pale yellow. In another, some globules were colorless, some yellow, some orange, some reddish orange, and these colors were found in globules of all sizes. Another adult had only pale yellow globules. The fact that the chitinous exoskeleton is brown on the thorax makes these variations in the internal color less conspicuous than they would be in any of the females, whose chitin is colorless over the entire body.

Green Apterous Parthenogenetic Female: Color in this form is due to minute green spherules, from 1.5 μ downward in size, contained in the body fluid. They are almost, or quite, as numerous as the red spherules of the pink apterous female, but the maximum size is a little greater. The color pattern is dependent on the distribution not only of the spherules, but of the internal organs, just as in the pink variety. Larger globules of fat (?) were uncommon in the green apterous parthenogenetic female, so that the general color is almost solely that of the green spherules.

Green Alate Parthenogenetic Female: In the winged parthenogenetic females the externally visible color is usually slightly yellowish green. The green color is due to many thousands of minute spherules similar to those of the apterous female described above. In addition to these are thousands of larger globules, up to 7 or 8 μ in the individuals examined, some of which are of a bright yellow color, others faintly yellow or colorless.

Green Oviparous (*Sexual*) *Female:* The young oviparous females of the green variety are very pale green. As they approach maturity they usually become yellowish, and at the height of their egg-laying period are commonly of a bright yellow. The color is found in multitudes of globules in the body fluid, ranging in size from barely visible up to 20 or 30 μ. In young individuals comparatively few of these are yellow, the rest colorless. In older females practically all the globules of a diameter of 4 or 5 μ or less are bright yellow, while the larger ones are nearly colorless. The increase in depth of yellow with age is due to the assumption of the yellow color by a larger number of the globules of nearly all sizes.

The newly laid eggs of this oviparous female are bright green, turning black later, as in the pink variety.

Green Male: This form is better styled the male of the green variety, since at no stage is it really green. The young male is either of a pink, dull pink or grayish or pale ocher color. The adult is internally yellowish or salmon colored, with brown chitin on the thorax, which modifies the general appearance. The color is due to hosts of globules in the body fluid, ranging from a minimum diameter of about 1.5 μ up to 3 or 4 μ in some individuals, or to 8 or 9 μ in others. In the pink young individuals, some of these globules are of a bright orange pink color, some colorless, with all gradations of color between these extremes. The brightest colors in the "green" male are brighter than the red of the spherules of the pink variety, but there are fewer of them, hence the duller pink color. The adult male contains usually a few orange spherules, some of paler yellow or salmon color, while probably about 80 or 85 per cent. are colorless. Apparently none of them are green.

Although the larger globules mentioned above in the descriptions of colors are tentatively regarded as composed of fat, or a fat-like substance, there is no certainty as to their composition. Whether there are two or more classes of substances included in the spherules is unknown, and the nature and source of the colors in them is likewise conjectural. These questions are under investigation.

Differences among Parthenogenetic Lines

The outstanding distinction between parthenogenetic lines in this species is that between the pink and the green variety. Each breeds true to its own color by parthenogenetic reproduction. Over ninety thousand individuals have been reared in my experiments, and there has not been one exception to the rule that pink parthenogenetic females produce only pink offspring, and green parthenogenetic females produce only members of the green variety. This confirms the results of Patch (1915) referred to above and removes any suspicion that the color difference is individual rather than varietal. The idea that parthenogenetic females of either color might produce offspring of either or both colors, if it existed, may have been due to the fact that individuals of the two colors live side by side on the same plant, that the males of the green variety are pink or nearly so in their young stages, and that, as described below, certain green parthenogenetic lines may have reddish-orange oviparous females.

Other differences between the pink and green varieties occur. Pink lines have always employed a longer time in parthenogenetic reproduction before entering the sexual phase than have the green lines. As a result the sexual period of the green variety is usually well advanced before that of the pink variety begins. One season the difference was so great that there was no overlapping of the sexual periods of the two varieties. Perhaps associated with this difference there is a difference in the rate of reproduction. In general, green individuals produce more offspring per day than do pink ones. By removing apterous pink and green aphids from plant to plant each day and ascertaining the number of offspring produced in each preceding 24-hour period, it was ascertained that the average number of offspring per day among the green females tested was 3.81 ± .073, while the mean for the pink females was 3.30 ± .064. These figures were obtained during a period of moderately warm weather one summer, and with the parthenogenetic lines then being used. They probably would not represent an annual average, nor an average of all lines, but do represent a difference between pink and green lines, which casual observation indicates is rather general.

Within the pink variety there are differences among lines, in the intensity and quality of the red color, due to variations in the number of colored spherules contained in the body fluid and to the nature of the color borne by them. Brick red, ocher red, orange vinaceous and flesh pink (Ridgway's *Color Standards and Nomenclature*) have been observed in different parthenogenetic lines.

Variations within the green variety are not conspicuous, so far as the parthenogenetic females are concerned, the extremes of color in the apterous individuals in the lines I have observed being dull green yellow and courge green (Ridgway). One much more striking variation was a green line in which the adult sexual females were, on part of the body, of a salmon-orange color, the rest of the body green. The color of the adult sexual female in most green lines is wax yellow. The parthenogenetic females of this line were not noticeably different from those of other green lines. Mottled pink and green individuals are mentioned by Patch (1915) as occurring late in the season. It is uncertain whether these were of the sort here described, which were obviously mottled owing to differences in the color and distribution of the contained globules, or whether they were green females containing pink male embryos or pink oviparous females containing green eggs. Any of these types should appear late in the season.

VARIATIONS WITHIN PARTHENOGENETIC LINES

Pink parthenogenetic lines have usually, in my experiments, become somewhat paler with age. The recorded color of one line when first collected in the spring was ocher red but in late summer was classed as orange vinaceous. Practically all lines varied in the same way, so that differences between lines observed in the spring were not diminished by the general fading of the color. Whether the fading is due to environment, such as a change in the quality of the food plant, or to something inherent in the animals themselves, is not known.

An extreme case of color change was observed in one pink apterous female which, when young, was distinctly pink, though somewhat paler than its sisters, and which later came to be of a translucent green. The genetics of such a change seemed more important than the physical basis of the color, so the female was kept as long as there seemed to be any chance that it would reproduce. It did not reproduce, however, and finally disappeared from the plant, presumably having died and dropped to the soil of the pot, where it could not be found. The nature of the color change is therefore unknown, but the failure to reproduce suggests that the individual was diseased.

INHERITANCE OF COLOR IN CROSSES

Matings were attempted in all possible ways in order to test the inheritance of color. These matings were made in some instances between males and sexual females of the same parthenogenetic line, in which case they may be spoken of as inbred matings. In other cases different green lines were mated with one another, different pink lines with one another, or pink lines with green ones.

In making matings, sexual females and males were confined on the same plant under a lantern globe, and the females, in case copulation occurred, subsequently laid their eggs on the stem or leaves of that plant. When the plant began to die those parts which bore the fertilized eggs were preserved. The eggs were given varying treatments, none of which resulted in more than a very low percentage of hatching. The first season in which fertilized eggs were obtained (1916) the remains of the plant with eggs on it were laid on the soil of a pot beside a young plant in the greenhouse. Only one egg in this lot hatched, requiring about two months in the egg stage. In one later season the plants with eggs on them were preserved dry for several months and then placed on soil by a growing plant; none of these eggs hatched. Another method was to place the plants with attached eggs in glass tubes, closed at the ends with gauze, and expose them for several weeks or months to the rigors of winter, under leaves and snow, after which they were brought to greenhouse conditions. Even with this treatment, which would appear to be nearly normal for the species in nature, the percentage of hatching was very low, many lines not producing a single viable egg. No single method in which the eggs were kept somewhat moist seemed to be particularly better than other methods which maintained moisture.

In all cases, a group of eggs placed beside a young plant to hatch was left there only several days and then moved to a new plant. No immediate examination of the plant was made, since it is extremely difficult always to discover a single young aphid on a potato plant with its hairy, curling leaves. Instead, the plant was kept several weeks, at which time many aphids would be present if any of the eggs had hatched.[2] This method made it possible that more than one egg hatched in the two- or three-day period during which the eggs remained beside one plant. It is not likely that this occurred very often, for

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[2] It is to be regretted that this method of obtaining aphids from fertilized eggs was not calculated to reveal the stem mothers. Although these aphids have apparently been obtained from fertilized eggs by earlier investigators, there does not exist, so far as I can find, a satisfactory description of the female so produced.

TABLE 1

SUMMARY OF MATINGS BETWEEN MALES AND SEXUAL FEMALES OF THE SAME GREEN LINES OF MACROSIPHUM SOLANIFOLIA RESULTING IN THE PRODUCTION OF FERTILIZED EGGS, AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1916	Potato	78	1	0	Potato
1917	Potato	276	0	0	....
1919	Rose	284	0	0	....
		461	0	0	....
		326	2	0	Potato
1920	Rose	641	0	0	....
		382	0	0	....
		567	4	0	Potato
		448	2	0	Potato
		490	0	0	....
1921	Rose	183	0	0	....
		265	2	0	Potato
		417	0	0	....
		314	2	1	Potato
		482	0	0	....
		334	3	0	Potato
		318	0	0	....
		427	0	0	....
		366	0	0	....
		282	4	0	Potato
		165	0	0	....
		325	0	0	....
		229	0	0	....
		403	0	0	....
1922	Rose	227	0	0	....
		84	2	0	Potato
		177	0	0	....
		351	1	0	Potato
		272	0	0	....
		55	0	0	....
		475	0	0	....
		269	3	1	Potato
1923	Rose	401	0	0	....
		762	4	0	Potato
		363	0	0	....
		499	0	0	....
		554	2	0	Potato
Total		12952	32		

the eggs were hatching so infrequently that only once in all the experiments did two successive plants used for one batch of eggs have aphids on them, and in no case were both pink and green aphids on one plant. Large numbers of plants could be used in this way without entailing prohibitive labor.

It is important to know whether covering the plants with lantern globes with gauze tops excludes foreign aphids. Macrosiphum is not one of the excessively restless species, and there is no internal evidence in the very long experiments testing the distinctness of the pink and green varieties that any contamination occurred. Nevertheless, numerous direct tests of the efficacy of isolation by lantern globes were made by covering plants having no aphids on them and setting them near infested plants. In no case did the aphids reach any of the plants thus guarded.

As was pointed out above, the percentage of hatching eggs was always small. Nevertheless, enough offspring have been obtained to indicate clearly the genetic nature of the color differences. The matings, which extended over the period from 1916 to 1923, are collected in tables 1 to 5, classified with respect to the type of mating. All information that is thought to bear on the questions discussed in this paper is given in these tables.

Attention is called to the fact that crosses between green and pink lines were made only with green females and pink males. This is owing to the fact, stated above, that the pink variety entered its sexual phase several weeks later than the green variety. In practically all lines the males appear before the sexual females, so that by the time sexual females of the pink lines were produced the males of the green lines were gone. Usually there were green line females when pink males appeared, and it was with these that the crosses were effected.

The twelve F_1 lines derived from the eggs in Table 5 were preserved for a time, as were also the two green lines from pink parents recorded in Table 4, in an at-

TABLE 2

SUMMARY OF MATINGS BETWEEN MALES AND SEXUAL FEMALES OF SUPPOSEDLY DIFFERENT PARTHENOGENETIC GREEN LINES OF MACROSIPHUM SOLANIFOLIA, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS, AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them.		First food plant of offspring
			Green	Pink	
1921	Rose	284	2	0	Potato
		424	0	0	....
		195	2	1	Potato
1922	Rose	782	1	0	Potato
		156	3	1	Potato
		445	0	0	....
		722	0	0	....
1923	Rose	557	1	0	Rose
		335	0	0	....
		681	2	1	Rose
		595	4	0	Rose
Total		5176	15	3	

tempt to secure further generations of parthenogenetic lines from them. Seven of the fourteen, including the two from pink parents, were lost in a period of depression in the winter of 1923, due probably to unhealthy food plants (potato). The four produced in 1922 (Table 5) went into the sexual phase the following summer, and their fertilized eggs were kept in moist air at ordinary temperatures, but none hatched. The remaining three lines from eggs produced in 1923 went into the sexual phase in February and March, 1924. Their eggs were at once subjected to moderately low temperatures out of doors and then brought into the greenhouse, but none hatched. This failure to hatch removes one important element in determining the mode of inheritance of color, and makes the explanation rest upon the data obtained up to the late fall of 1923. Fortunately, however, there can be little doubt regarding the general nature of the genetics of color.

TABLE 3

SUMMARY OF MATINGS BETWEEN MALES AND FEMALES OF THE SAME PINK PARTHENOGENETIC LINES OF MACROSIPHUM SOLANIFOLIA, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1921	Rose	37	0	0	....
		97	0	0	....
		22	0	0	....
		104	0	0	....
		34	0	0	....
1922	Rose	188	0	0	....
		65	0	0	....
1923	Rose	419	0	1	Rose
		289	0	0	....
		58	0	0	....
		456	0	0	....
Total		1769	0	1	

The explanation of the inheritance of color remained uncertain until the results from the matings of 1923 were obtained. From the first, inbred or crossbred lines produced mostly green individuals, but occasionally (beginning 1921) a pink one. The crosses between the two color varieties have yielded only green lines. The first hatchable egg of a pink line (obtained in 1923, Table 3) yielded a pink individual. All the foregoing results could have been explained by assuming a single factor difference between the color varieties, with green dominant. However, in the 1923 results were two cases (Table 4) in which crossbred pink lines gave green offspring. To explain all these results it seems necessary to assume that color is dependent upon multiple factors; and that while the factors possessed by the pink line are largely those producing pink color, it is nevertheless possible to obtain from the pink lines (at least by crossing different lines) the right combination of factors for green color. This assumption is moreover in better keeping with the variability of color exhibited by the various lines, especially in the pink variety.

If the conclusion that multiple factors are involved in the production of color is correct, further progress will probably require a prohibitive amount of labor, unless some means is found of inducing a larger proportion of fertilized eggs to hatch. The next desirable steps would be to ascertain how many factors are concerned, whether they are of one kind or several, and possible differences in the relations of factors to one another. Thousands of hatchable eggs might be required to attain these ends. Whether an increase in the hatchability of eggs may be artificially effected is uncertain, since there is no necessity for concluding that more of the eggs hatch even in nature. In a closely related species, *Macrosiphum granarium*, Phillips (1915) has found a similar high mortality of the fertilized eggs, which may be a normal condition in this genus and present insurmountable obstacles to further work.

RATE OF REPRODUCTION PROBABLY NOT INDEPENDENTLY INHERITED

As previously stated, the green lines tested produced a larger mean number of offspring per day than did the pink lines being reared at the same time, and casual observation leads me to believe that this is a general difference. Inheritance of this difference could not be studied in a wholly satisfactory manner, since in every case, by the time the F_1 line appeared, the green parent line was no longer in existence because it had gone completely into the sexual phase. No direct comparison with the green parent could therefore be made. In the case of the eight F_1 lines derived from crosses in 1923 in Table 5, the pink parent line from which all these were descended was still alive when the F_1 green lines started. Unfortunately, five of these lines were lost before the rate of reproduction was determined. The three remain-

TABLE 4

SUMMARY OF MATINGS BETWEEN MALES AND FEMALES OF SUPPOSEDLY DIFFERENT PINK PARTHENOGENETIC LINES OF MACROSIPHUM SOLANIFOLII, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS AND THE OFFSPRING FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1923	Rose	286	0	1	Rose
		394	0	0	....
		427	1	1	Rose
		580	1	1	Rose
Total		1687	2	3	

ing ones gave, at that time of year (mid-winter), daily output of offspring as follows: 2.82 ± .088, 2.91 ± .090, and 2.95 ± .094. The daily output of the pink parent line, then seven months old, at the same time was 1.53 ± .075.

A more important comparison may be made in the case of the two green lines derived from pink parents in Table 4, whose rate of reproduction was determined before the lines were lost. These two lines produced offspring at the daily rate of 2.56 ± .061 and 2.87 ± .072, respectively. The particular pink lines from which these were descended had been discarded to make room for other experiments, but the pink line mentioned in the preceding paragraph was being reared and was producing at this time, as stated above, 1.53 ± .075 offspring per day.

The difference between these two hybrid green lines and the (perhaps) unrelated pink line with which they were compared is greater than the difference between green and pink lines given earlier in this paper. Possibly this greater difference is due to the fact that the lines were not of the same age when compared. The hybrid green lines were about one month old, the pink one about seven months old. Whether age of the line or

TABLE 5

SUMMARY OF MATINGS BETWEEN MALES OF PINK LINES AND FEMALES OF GREEN LINES OF MACROSIPHUM SOLANIFOLII, RESULTING IN THE PRODUCTION OF FERTILIZED EGGS AND THE OFFSPRING OBTAINED FROM THESE EGGS

Year eggs were produced	Source of line producing eggs	Number of fertilized eggs	Number of eggs hatching and color of offspring from them		First food plant of offspring
			Green	Pink	
1922	Rose	75	0	0	....
		126	0	0	....
		88	1	0	Potato
		255	3	0	Potato
		66	0	0	....
1923	Rose	879	4	0	Rose
		1063	4	0	Rose
Total		2552	12	0	

long-continued uniform conditions of life could cause such a difference is unknown.

Most of the other F_1 lines were reared through a number of generations to test their uniformity of color, but were discarded, before the rate of reproduction could be ascertained, on account of the labor involved.

ORIGIN OF PINK LINES IN NATURE

That pink lines can be derived from green parents or from pink parents, under greenhouse conditions, has been demonstrated. Whether they may also be descended from one green and one pink parent has not been shown, though such a result would seem not improbable. In nature it is questionable whether all these possible sources of pink lines are ever available in our latitude. The parthenogenetic phase of the pink lines lasts so long that it is doubtful whether the summer season in this part of the world will suffice for a well-marked sexual phase, unless in exceptionally late autumns. Under these circumstances the pink lines of each year must be derived wholly or in part from the green lines of the preceding season.

SPECIES RELATION OF THE TWO VARIETIES

Probably no systematic zoologist would entertain a proposal to place two color varieties of any insect in different species, if the chitinous structures of both were identical as they are in *Macrosiphum solanifolii,* whether he knew anything of the genetics of color or not. A geneticist, however, might be inclined to give them separate specific recognition if it could be shown that neither variety produced the other. Any one could have determined, with a little trouble, that during the parthenogenetic phase the two varieties are absolutely distinct. Whether they are thus distinct in bisexual reproduction was unknown until the work reported in this paper was done. Since, as here shown, either variety can produce the other, and under natural conditions the pink variety probably usually and perhaps always comes from the green variety, even a geneticist could not maintain that the varieties constitute different species.

ALTERNATION OF FOOD PLANTS

Macrosiphum solanifolii is very tolerant of its food, having been discovered on scores of different kinds of plant. It is obvious, however, to any one who has followed the species through its entire cycle that the great majority of plants which may properly be termed its hosts are tolerated only by the sexual female. The sexual female comes to rest on almost any plant on which it happens to be placed, and commonly remains long enough to be regarded as having "settled." Whether it will lay its eggs on most of these is unknown, but I have observed its eggs on several plants from which the parthenogenetic forms always departed immediately when placed on them. If, therefore, plants were designated as proper hosts only if all forms of the species lived upon them, the list would be greatly reduced. But even with this restriction, the host plants of *Macrosiphum solanifolii* would be much more numerous than for the majority of aphid species; and the species has become a serious pest on plants as different as potatoes, kale, spinach and egg-plant.

Nevertheless, there is in the literature a tendency to describe this aphid as alternating between the rose and the potato. Part of this tendency is no doubt due to extending to all aphids a conception which is strikingly correct for certain species which are strictly limited to two kinds of food plant, but is partly justified by observations in nature. Patch (1915) refers to the rose and the potato as "two favorite food plants," but recognizes that the species is at home on many others. She finds, nevertheless, that the potato is the common summer host, that potato plants are deserted in late summer and that the rose is favored by the "fall migrants." In the spring the first individuals are found on the rose, but migration to potatoes occurs in early summer, and one infers from her description that the rose is free from them during the summer. Regan (1917) also describes a sort of alternation between host plants, but in very general terms.

Observations recorded in this paper indicate that whatever alternation between hosts occurs in this species has no significance; that is, it indicates nothing whatever concerning the inherent nature of the insects or their cycle. The stem mother, immediately upon hatching from the fertilized egg, feeds upon either the rose or the potato, whichever is available, as is shown in Tables 1 to 5. They continue to live on either plant so long as tender tissues are available on them, and may be transferred from either one to the other at any time the year round. There is little difference between winged and wingless females in this respect, for both commonly remain, without confinement, on a plant so long as conditions are favorable, and both migrate freely when conditions are unfavorable. The winged females presumably travel farther than wingless ones, but there is little to indicate that they are more successful in finding suitable new hosts except in cases where large regions are rendered uninhabitable. In particular, there is nothing to show that the migration from the rose to the potato is done only or even chiefly by the winged female. The sexual forms live with equal readiness on either plant, and have laid their eggs on potatoes, while Smith (1919) reports finding the eggs of this species on the rambler rose.

A rough alternation between potato and rose does, however, occur. My observations in this region support those of Dr. Patch in Maine. The species is nearly always to be found on roses earlier than on potatoes. During the summer it is usually absent from the rose, but regularly occurs on the potato at that time. I have found sexual forms on the potato in late summer or early autumn, but never on the rose. How or when the rose again becomes infested is uncertain. This approximate alternation, in time, between the rose and the potato does not always occur, for sometimes aphids may remain on the rose all summer. In 1922 they were found on the same stem of a certain rosebush continuously from May 18 to September 9. Winged individuals were usually present during that time, at least after the first several weeks, although it is not known that any one winged individual remained very long.

How, then, is the usual temporal alternation between the rose and the potato accounted for? It is probably true that young growing rose shoots are available earlier in the season than are potatoes, which may account for the earlier discovery of them on roses. Their disappearance from the rose in most years is, presumably, due to rain and wind. The aphids are easily jarred off their food plants. On the rose they live only on the stems, near the tips where the stem is still tender, or on the very young leaves, and never on the older leaves, which are thin and papery and would probably furnish little food. The tips of the stems are whipped about violently by strong wind, and are nearly always so located as to be beaten by raindrops. I have repeatedly observed that rose tips so thickly covered with aphids that there appeared to be no room for any more were, immediately after a rain- or windstorm, completely or practically bare. The inimical effect of rain upon aphids has been noted by many writers (Patch, 1915; Phillips, 1916; Houser, Guyton and Lowry, 1917; Britton, 1918; and others). On potatoes, the aphids are not limited to the stems, but very often settle on the under surface of the leaves. The leaves are usually concave downward, which probably affords considerable protection from rain. Moreover, the potato plant is hairy, which may make it more difficult to dislodge the aphids. The aphids do collect in great numbers on the tender growing stems of potatoes, just as on the rose, and I have observed that a heavy rain or wind may largely dislodge them from such places; but there are usually individuals under the leaves of such plants by which the whole plant may soon again be stocked.

That the disappearance of the aphids from the rose in summer is due to the mechanical agency of rain and wind seems to be confirmed by the weather record of the one season, 1922, in which I found them on the rose all summer. The summer of 1922 was unusually dry. The total rainfall in Ann Arbor during the five months, May to September, was only 10.18 inches, as compared with 16.37, 17.72, and 18.31 inches for three other recent years. The maximum daily rainfall is of interest in this connection. The last rain of any importance in the spring of 1922 was on May 19, 1.38 inches, probably before stem mothers had ceased to hatch from overwintering eggs. The largest single rain in June was on the 10th, 0.66 inch; in July, on the 12th, 0.38 inch; and in August, on the 29th, 0.63 inch. The first considerable rain in the fall was on September 11, 1.38 inches. September 9 was the last day on which aphids were found on the rose; no further observations were made until just after the rain of the 11th, and then the aphids were all gone.

SUMMARY

(1) *Macrosiphum solanifolii* exists in two varieties, pink and green, whose color is due to globules of different

colors and sizes contained in the body fluid. The several forms belonging to each of these varieties are briefly described.

(2) Pink lines differ from one another somewhat in color and green lines differ from one another, owing to differences in the color and number of globules contained in the body fluid.

(3) The pink color of a line commonly fades somewhat during the season; the cause of the change is unknown.

(4) In green lines the sexual (oviparous) female is most often wax yellow in color, sometimes greenish; in one line, however, the sexual females were mottled with salmon-orange on the abdomen.

(5) The sexual phase usually appears later in the season in pink lines than in green lines.

(6) Pink parthenogenetic females produce only pink offspring. Green parthenogenetic females produce only members of the green variety, some of which, however, are not green.

(7) Very few of the fertilized eggs of sexual females hatch. No treatment capable of increasing their viability has been discovered.

(8) Sexual females of a green line mated with males of the same line produced mostly green offspring, but two of them were pink.

(9) Sexual females of one green line mated with males of a supposedly different green line produced offspring that were mostly green, but several pink ones were included.

(10) Pink sexual females mated with males of the same line produced only pink offspring. Since there were only two of these, it is thought that green offspring might also be obtained from such parents.

(11) Pink sexual females mated with males from other pink lines produced both green and pink offspring.

(12) Sexual females of green lines mated with males of pink lines produced only green offspring, though it is thought that pink offspring may occasionally be produced by such parents.

(13) The results of the above matings appear to indicate that color in these aphids is due to multiple factors. This assumption also fits the fact that lines differ among themselves in color, even within one color variety.

(14) The pink lines studied produced somewhat fewer young per day than did the green lines.

(15) This difference in the rate of reproduction exhibited by the two varieties seems to be associated with the color, and not to be independently inherited.

(16) Under natural conditions the pink variety probably descends chiefly, if not wholly, from the green variety each year.

(17) The approximate alternation of this species between the potato and rose is probably due to the earlier growth of shoots of the rose than of the potato and to removal of the insects from the rose by rain and wind, and does not indicate any inherent property of the aphids or of their cycle. Any form belonging to the species may live and reproduce on either of the principal host plants at any time of year.

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THE FACTORS OF INHERITANCE AND PARENTAGE AS AFFECTING THE RATIO OF ALATE TO APTEROUS INDIVIDUALS IN APHIDS[1]

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THE influence of parentage on aphid forms has been noted by Shull (1918), is very apparent from the earlier results obtained by Gregory (1917) and has been recently confirmed by Wadley (1923). The results of all three of these workers appear to be entirely in accord in two respects, *i.e.*, in regard to the existence of a tendency for winged parents to produce wingless offspring and for wingless parents to produce winged offspring. Shull also noted that winged males were the progeny of apterous mothers and that wingless oviparous females were the offspring of alate females. These observations caused him to relate the phenomena with Riddle's theory of sex. He regarded the winged agamic individuals and the males as the forms of high metabolism and low energy content and the wingless agamic females and the oviparous females as forms of low metabolism and high energy content.

Miss Gregory (Gregory, 1917), whose results were published a year earlier than those of Shull, came to the conclusion that the primary factor in determining the development of wings was the food supply. Her work was done with the green pea-aphid *Microsiphum destructor.* In one series of experiments, "G series" (Gregory, 1917, p. 302, table IV), it was found that winged parents even when starved produced no winged offspring.

Wadley observed the effects of various factors on progeny of different parentage and grandparentage. His

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[1] Contribution from the Department of Zoology and Entomology, Iowa State College.

work in the main confirms that of Gregory and of Shull in regard to this tendency of parents to produce a higher percentage of offspring of the reverse form. In regard to the effect of wingless mothers on their progeny Wadley's results are not so confirmatory.

Previous to the work of Shull and of Miss Gregory the present writer (Ewing, 1916) had shown that temperature was an important factor affecting wing production in the apple-grain aphid, then known as *Aphis avenae* Fab. This work on temperature was only very briefly reported in my larger paper (Ewing, 1916) dealing with the inheritance and biology of the species. Even in a later paper (Ewing, 1917) little was added in regard to the results obtained pertaining to general biology.

Here further results are presented in which certain data and conclusions are given in regard to the effect of inheritance and parentage on the ratio of alate to apterous forms. Also in this paper an attempted explanation is given in regard to the phenomenon of parental and ancestral effect on aphid forms which for many years I have had in mind but which I have hesitated to give for lack of sufficient support by the way of experimental data. The recent publication of Wadley's excellent and comprehensive paper (1923) dealing with the identical species with which I worked has given so much of the desired data that it appears profitable to give this explanation.

The explanation is: It appears that in the case of the apple-grain aphid, and presumably in similar species of aphids, there is a type of inheritance not dependent upon the germplasm (or at least the chromosomes) but which modifies the expression of somatic characters which are dependent upon the germinal elements in the following important ways: (a) through the general metabolism of the individual which in turn is affected by its environment; (b) in such a way as to produce cumulative and hold-over effects from generation to generation; (c) through cumulative effects to produce an unbalanced state of metabolism which eventually rights itself by returning towards the mean of the race. The term physiological inheritance might be applied to this parental and ancestral transmission of cumulative and oscillating metabolic inertia.

THE EFFECT OF TEMPERATURE

In 1916 (Ewing, 1916) the effects of temperature on the production of alate or apterous individuals in the apple-grain aphid, then known as *Aphis avenae* Fab., was shown. Lots of 10 each of reproducing wingless parent individuals were placed at different times for two days in a temperature cell at the following constant temperatures: 60° F, 70° F, 80° F, 90° F. At the end of two days these parents were removed and their progeny, thus affected, on the average for a day before their birth at the constant temperature at which they were born, were allowed to grow at that temperature until they either died or reached maturity.

In performing this experiment only wingless mothers with a long line of wingless ancestors were used.[2] Thus the effects that may have been produced by having a mixed parentage (some wingless and some winged) were avoided. Particular attention was given to having as uniform nutrition as possible, all individuals were placed on wheat shoots reared fresh for each experiment from the same seed bag and no individuals were started on any plant that had unfolded its leaves. In order to avoid, if possible, any effects on metabolism due to humidity, the soil in the breeding cell was kept moist, thus insuring, it was believed, a humidity above 37 per cent.; a degree of humidity which should give no change in the rate of metabolism (Headlee, 1914). During the whole of the experiment the aphids, and of course the wheat shoots on which they were placed, were kept in greatly subdued light. The specially constructed asbestos-lined "oven" was lighted only by a minute opening which faced the side of a room many feet from the sunlight and the single outside window. And finally, the same mixed sand and soil was used for growing all the wheat plants.

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[2] In looking up my records of the parentage of individuals I find that the exact grandparentage of lots used in the 60° F. experiment is not recorded; however, they were from a fraternity that included but two-winged females and were certainly almost entirely if not entirely of wingless grandparentage.

As a result of these experiments the following points in regard to temperature were determined:

(1) That the optimum temperature for the development of the wingless forms was near 65° F.

(2) That this temperature was shown to be a very important factor affecting the ratio of wingless to winged forms produced.

(3) That the percentage of winged individuals produced increased as the temperature varied both ways from the optimum for the production of wingless forms, which optimum was very near, if not the same as, the optimum for greatest metabolic activity (velocity of chemical reaction (?) Sanderson, 1910).

Further, as a result of these temperature experiments on individuals of a single strain, of a single race with a known parentage for 16 generations or more, and of a uniform ancestry of wingless parents, it was decided to test further the effects of temperature by its regulation in the small room in which the experiments were conducted so as to avoid as far as possible large variations from the optimum of 65° F. An endeavor was made to see if many winged individuals would be produced under these conditions. In this second experiment, which was in the nature of a compound one, for I was selecting for a definite morphological character in order to test the pure line theory of inheritance, no special precautions were taken in regard to the control of other factors than temperature. But, however, it is to be noted that each succeeding generation was started on tender shoots, as had been my practice for months before the temperature experiments were conducted.

As a result of this second experiment I was able to propagate the line for 20 generations without the appearance of a single winged individual. In the 16 generations obtained before the temperature experiment was started, winged forms had appeared in 6, and in the case of one generation constituted all the individuals, and in the case of another generation a majority of the individuals of the fraternity.

The results obtained in regard to the effects of temperature were presented in less than 2 pages in my 1916 paper. At the time the experiments were made considerable importance was attached to the results, but because the number of individuals used was small it appeared advisable not to give them undue importance in the printed form. Viewed in the light of the subsequent work by others, and particularly in the light of Wadley's experiments on the same species, the experiment has a much greater significance. I think that it should be particularly valued; as it is, apparently, the only experiment that has been performed on the effects of an environmental factor in influencing the production of apterous or alate asexual forms of plant lice, in which individuals were used that had the same parentage (in regard to the presence or absence of wings) for a long series of generations previous to the test of controlled factors.

WADLEY'S RESULTS

Before considering further my experiments on parentage, temperature, etc., it is best here to review Wadley's results along the same line. Wadley (Wadley, 1923), carried out a series of experiments on the effect of temperature on the dimorphism of asexual females using the temperatures of 62° F, 65° F, 70° F, 72° F, 75° F and 80° F. In his experiments he used individuals of four kinds of parentage; those with both grandparent and parent apterous, those with grandparent alate but parent apterous, those with grandparent apterous but parent alate and those with both grandparent and parent alate.

In regard to his results: In general they parallel those of mine, as he states in his paper; however, there is this very important difference. The percentages of alate individuals produced in his experiments is much lower than in mine. In explaining this difference he suggests that it might be attributed to a possible lack of nutrition control in my experiment. This, however, could hardly be the case, as I made a particular effort to see that the individuals had the same kind of food.

When I first observed his "parallel" comparison of our results (Wadley, 1923, p. 291), it was difficult for me to explain the differences, but in looking at his table on the bottom of page 287 (Wadley, 1923) it became apparent. The figures given for his experiments on page 291 (Wadley, 1923) are averages for all individuals regardless of parentage. My experiments were with individuals having a long line of apterous parents. (However, this fact was not clearly stated in the 1916 paper.) When we compare his results for individuals that had both parents and grandparents apterous with my results there is a much greater conformity.

In order to further analyze the effects of parentage I will give in this paper the results obtained by both Wadley and myself in the form of a plotted curve, but first I shall present the pedigree of the individuals used in my temperature experiments. This is here given (Table I) for the first time.

Only wingless forms were used as parents for all the individuals born and reared in the constant temperature experiments.

In the pedigree chart it is seen that in the case of three of the four sets of ten individuals used as parents in the constant temperature experiments the parentage of each ancestor for many generations back is known and each of these was a wingless individual.

In the case of one set of ten parent individuals, the set used for the 60° constant temperature experiment, the exact parentage of these mothers (those from whom the individuals at the constant temperature were born) is not known. However, the grandparents are known to

TABLE I

Pedigree of Aphids used in Temperature Experiment

Number of generation of isolation	Key number and status as to wings of ancestral parent	Date of selection of parent and the isolation of her young offspring	Number of offspring	Status of adult offspring. Wingless	Status of adult offspring. Winged	Died or lost in infancy
I	10_{39} 9 wingless	Aug. 4 (1914)	17	0	15	2
II	11_{40}10 winged	" 17	3	3	0	0
III	11_{41} 1 wingless	" 28	6	1	0	5
IV	11_{42} 1 "	Sept. 6	9	8	0	1
V	11_{43} 3 "	" 13	13	9	0	4
VI	11_{44}10 "	" 20	10	9	0	1
VII	11_{45}10 "	" 27	14	7	1	6
VIII	11_{46} 3 "	Oct. 4	12	5	4	3
IX	11_{47} 4 "	" 11	21	14	0	7
X	11_{48} 5 "	" 18	13	8	1	4
XI	11_{49}11 "	" 25	16	13	0	3
XII	11_{50} 1 "	Nov. 1	25	20	0	5
XIII	11_{51}12 "	" 8	28	22	0	6
XIV	11_{52}10 "	" 15	26	22	0	4
XV	11_{53} 8 "	" 22	11	9	2	0
XVI	11_{54} 3 "	" 29	28	11	15	2
XVII	11_{55}25[3] "	Dec. 6	16	3	10	3
XIX	11_{56} 8 "	" 13	19	10	6	3
XX	11_{57}11 "	" 20	18	18[4]	0	0
XXI	11_{58}11 "	" 31	5	5	0	0
XXII	11_{59} 3 "	Jan. 7 (1915)	21	16	0	7
XXIII	11_{60}15 "	" 13	15	14[5]	0	1
XXIV	11_{61} 6 "	" 20	11	10	0	1
XXV	11_{62} 3 "	" 27	13	13	0	0
XXVI	11_{63} 2 "	Feb. 3	18	15	0	3
XXVII	11_{64}15 "	" 8	8	8	0	0
XXVIII	11_{65} 1 "	" 13	14	13	0	1
XXIX	11_{66}12 "	" 19	15	15[6]	0	0

[3] Parents of 10 mothers used in 60° constant temperature experiment among fraternity of this individual.

[4] Ten of these 18 wingless individuals used as mothers for 70° temperature experiment.

[5] Ten of these 14 wingless individuals used as mothers for 80° temperature.

[6] Ten of these taken for 90° constant temperature experiment.

have been among the individuals of a fraternity of 11 individuals only two of which were winged. That some of these 10 mothers came from a winged form is possible but not probable. The chances are against it. But even if some of these did come from a winged parent the number could not be large enough to affect seriously the results of the experiment.

When we compare the results obtained in my temperature experiments with individuals of apterous monomorphic parentage with the parentage control temperature experiments of Wadley we get the following results:

Percentage of Progeny Alate at all Temperatures

From alate parents and grandparents (Wadley)	0 per cent.
From alate parents only (Wadley)	2.28 " "
From apterous parents only (Wadley)	6.07 " "
From apterous parents and grandparents (Wadley)	9.24 " "
From apterous parentage for 16 or more generations (Ewing)	30.38 " "

Thus it is noted that there is a summation effect of parentage on the offspring of a generation of aphids depending upon the number and nearness of the ancestors of either form. Two generations of alate ancestors gave 0 per cent. of alate offspring. Where the parent only was alate a percentage of 2.28 was obtained. The immediate parent being an apterous individual gave a percentage of winged offspring of 6.07; both parent and grandparent being apterous gave a percentage of winged progeny of 9.24; apterous parentage for 16 or more generations gave a percentage of 30.38 of alate offspring.

When these results are analyzed as to temperature effects and the whole plotted in the form of curves, the effects are more easily comprehended. These plotted results (Table II) show in an excellent manner not only the striking effects of temperature but the equally striking effects of parentage on the ratio of alate and apterous individuals produced.

Undoubtedly there were several factors, such as quality of food, nature of soil used to grow wheat plants and

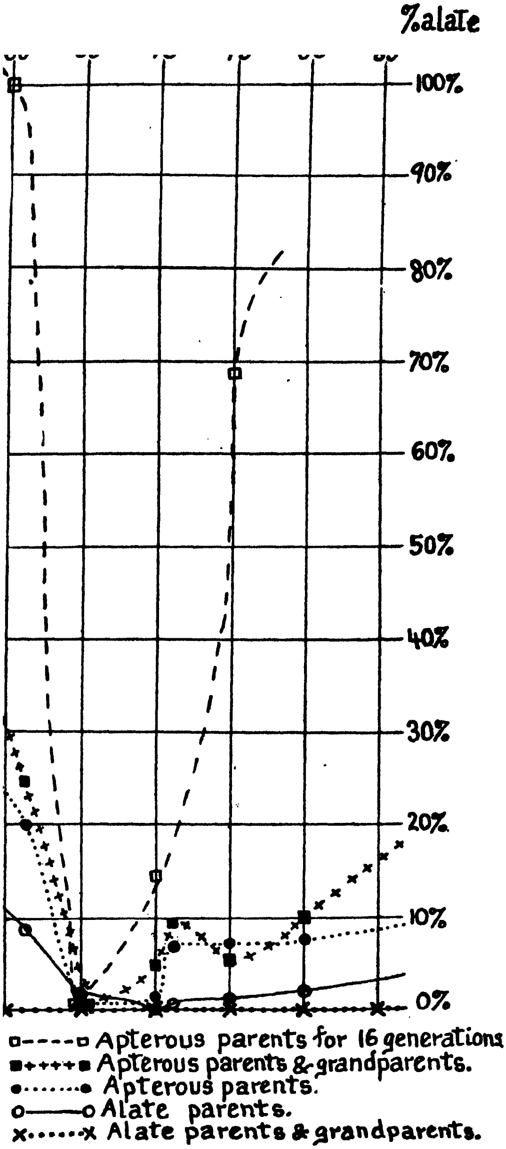

FIG. 1. Plot showing effects of temperature and parentage on the percentage of alate individuals produced (given in column to right) of apple-grain aphid. (Data from Wadley and from Ewing.)

light and evaporation that were quite different in these experiments, but their remarkable conformity in regard to both temperature and parentage is very striking.

THE EFFECT OF TEMPERATURE ON THE PRODUCTION OF SEXUAL FORMS

The apparent effect of colder weather in the autumn on the production of the sexual forms of plant lice in temperate climates was noted by several entomologists many years ago. In more recent times various workers have found that species which normally produce the sexes in the colder sections of the temperate zone reproduce without them from year to year, when these same species occur in tropical or subtropical countries.

Uichanco (1921) has accumulated much evidence in regard to the effect of temperature on the production of the sexes. He claims that but few aphids of any species produce the sexes in the tropics. He cites the experience of Goot, who after three years of biological work on the Aphididae in Java failed to find any sexual forms in that island. Speaking of his own experience he states that:

> Likewise, several years of casual observation and collecting in the Philippine Islands by me failed to disclose the male and oviparous female forms. So far as I am aware, the occurrence of amphigonous aphid individuals in any other tropical country has not been definitely reported.

While in general it is true that the majority of aphids do not produce sexual forms in the summer in temperate countries, yet in a few species the sexes have been reported even for the months of July and August (Patch, 1920, p. 162). These exceptions, however, could hardly be used for supporting the hypothesis advanced by some that temperature has little or no effect on the production of different forms of aphids. The observations made, not only in the tropics but also in the temperate regions, undoubtedly indicate that temperature is a very great factor affecting the production of different forms.

As has been stated previously in this paper the apple-grain aphid reproduces on the Pacific Coast without the appearance of the sexes. Relative to this statement it would appear pertinent to record the following observation made at Corvallis, Oregon. During the winter of 1913–14 large numbers of Coccinellidae were carried over for various purposes to the following spring. In order to secure for these Coccinellids an ample food supply a screened-in, out-of-door bed of wheat plants was infested with the apple-grain aphid during 1913. Here the aphids multiplied by the thousands and passed the entire winter without a single sexual form or egg being found. The bed of wheat was several feet long and almost as wide. The wheat plants grew until finally they were almost a foot high.

Later (1914) at Ames, Iowa, the effect of temperature was tried. On December 11, the offspring of 11_{56} 7w, a winged individual, were taken with the potted wheat plants on which they were feeding and placed on a window-sill and the window closed. The nymphs grew slowly, but with the coming of temperatures near zero Fahr. were all killed.

Wadley (1923) attempted to produce the sexes in the apple-grain aphid but was unsuccessful. He states (p. 302):

> With the species used by the writer, cold, hunger or long-continued parthenogenesis did not produce the sexes and artificial colonization on the winter food-plant was ineffective. Just what combination of factors will produce the sexes is yet to be found.

Experimentally, Davidson (1924) has shown that lowering of the temperature may cause the appearance of sexes in *Aphis rumicis* L. After propagating a pure line of this species from November to January 15 under artificial light conditions and at higher temperatures, during which time only agamic individuals appeared, he found that after the placing of the individuals under conditions of lower temperature sexual forms appeared in the generations from February 10 to June 10. Davidson came to the conclusion that "it seems highly probable that sunshine, temperature and length of day are influential factors" affecting the appearance of the sexes and affecting the ratio of agamic to gamic individuals.

Effects of Nutrition

Early in his work with the apple-grain aphid the writer observed that those nymphs which settled upon the expanded wheat leaves or those that fed upon withering plants were more frequently winged than those that fed upon the succulent shoots. Later the effect of this type of feeding appeared to be demonstrated incidentally in one of the heredity experiments.

In isolation 11, after five generations of individuals in which not a single winged form appeared, suddenly in the seventeenth generation 15 individuals out of 26 became winged. One of the wingless individuals of this fraternity was selected as a parent. From it even a larger percentage (10 out of 13) were winged.

In order to explain this sudden appearance of winged forms the conditions of their rearing were examined. First, temperature was suspected, but it was found that the average daily mean was 73.04° F. This was not very far from the optimum and should have given only a small percentage of individuals winged. Then it was noted that due to the lack of proper watering of the soil the wheat plants had been allowed to wither and shrivel. This was the only unusual condition that had been noted and was very probably the cause of the sudden appearance of these winged forms.

Taking advantage of this experience greater care was given the plants and not only were they kept well watered, but the most of the first formed leaves were clipped off to prevent the aphids from feeding on the harder tissue of the leaf surface. As a result of these precautions several generations were again procured without the appearance of a single winged form.

The effects noted in this experiment were later verified in a way in the starvation experiments of Miss Gregory (1917) and of Wadley (1923), the later authority using the same species as the writer. So impressed was Miss Gregory with the effects of starvation that she assigned to nutrition the major rôle in the altering of the ratio of form in aphids.

In regard to the relative influence of temperature and nutrition on the production of winged agamic aphids there has been a wide divergence of opinion. Miss Gregory states:

> In conclusion it seems most probable that the lessening of the food supply is the primary factor in determining the development of wings in the offspring of wingless mothers. The wing anlage appears to be present in all the parthenogenetic females and depends directly upon the condition of the food supply of the mother for its stimulation or suppression of development.

On the other hand, some would hold that nutrition is of much less or even minor importance. Thus Baker and Turner (1916), working with *Aphis pomi* De Geer, found that lack of proper nutrition did not cause the winged forms to appear. They state (p. 976):

> The theory has been frequently advanced that the production of winged forms during the summer is due to a lack of sufficient nourishment for the insects. In some cases the wording of this theory is modified by the statement that winged forms appear on plants which are very heavily infested. The writers' results are a flat contradiction of this theory for this species. As has been stated previously, in handling the insects the writers always transferred the mothers to new plants, rather than the progeny. In this way several consecutive generations were reared on one plant. Thus the effect of poor or good food would be accentuated. Yet the winged forms were never obtained in series of small, poorly fed insects, but occurred frequently in well-nourished series.

Wadley, working with the apple-grain aphid, found, (page 297) that:

> A high percentage of winged forms was produced by starvation at all temperatures tested, but greater difficulty was encountered in producing alate aphids at 65° than at other temperatures.

SUMMARY IN REGARD TO FACTORS AFFECTING PRODUCTION OF FORMS OF PLANT LICE

Up to the present we have but little experimental evidence in regard to the factors which influence the appearance of sexual forms of plant lice. This evidence, such as it is, indicates that the factors affecting the appearance or percentage of sexual forms are similar to those affecting the ratio of the different forms of agamic individuals.

In regard to the factors affecting the ratio of winged to wingless agamic females much experimental evidence has been produced showing that there are several of these and that definite results can be obtained in the laboratory by modifying any one of them.

The percentage of either one of the agamic forms produced in any fraternity depends first of all to a very large degree upon parentage and ancestry. Temperature is a very important factor, in certain species, at least. For the production of wingless forms there is an optimum temperature, and a variation from this optimum in either direction causes an increasing number of winged forms to appear. At the optimum temperature for the production of wingless individuals there is a tendency of succeeding generations to approach 100 per cent. as a limit for this agamic form, and at such a temperature other factors lose most of their effect. The limitation of the food supply is of much importance in the production of winged forms, particularly at temperatures far from the optimum. The nature of the food from the standpoint of chemical composition affects the ratio of the two types of agamic individuals. Indirectly, probably, light and evaporation are of considerable importance as form-producing factors. Both of these, however, are very intimately associated with temperature. Light alone may affect either the aphids directly or indirectly through its effect on the growing plants on which the aphids feed.

Among the outstanding factors that have been demonstrated experimentally as affecting the ratio between the two agamic forms of aphids are inheritance, temperature and nutrition, and the relative importance of each of these appears to depend on the conditions imposed by the combination of all other factors. Either inheritance, temperature or nutrition may under certain conditions become the dominant or even the controlling factor.

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HEREDITARY ANEMIA IN MICE AND ITS RELATION TO DOMINANT SPOTTING

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CUÉNOT, in 1905 (2), first pointed out the absence of homozygous individuals among yellow mice, and subsequent investigations have shown the existence of a similar lethal factor in mice of the dominant white variety. Many attempts have been made to account for this deficiency, but it was not until 1910 that Castle and Little (1) attributed the lack of homozygous yellow mice to the lethal action of Y genes in the duplex condition. They presented convincing evidence that "the yellow egg which by chance has met a yellow sperm has its career ended thereby." Studies by Kirkham (5) have showed that some ova die after implantation, and these he identified as the missing homozygous yellow individuals. His work was later confirmed and extended by Ibsen and Steigleder (4). All these investigators agree that homozygous yellow mice are produced but die during early embryonic life, the exact cause of death remaining wholly undetermined.

In 1915 Little (7) revealed the presence of a lethal factor in the dominant white strain of mice. This factor associated with dominant white spotting he found to be genetically distinct from the lethal factor associated with yellow coat color. In 1919 (9) he presented evidence to show that while genetically distinct the two lethals produced the same phenotypical effects, the young in both cases dying soon after implantation.

In the course of breeding experiments with dominant white mice in this laboratory the appearance of aberrant offspring was noted. The work embodied in this paper was undertaken at Professor Danforth's suggestion as a first step towards an understanding of their genetics and embryology.

Since this study was begun, Detlefsen (3) reported that "in strains of black-eyed [dominant] white mice a type of young has occurred rather frequently, which invariably dies one to three days after birth. Their appearance is distinctive and they can always be identified, since they are about one half normal size and present a very white and bloodless appearance. Their occurrence suggests that the homozygous black-eyed white (which is lethal as in the case of the homozygous yellow) may perhaps develop in some instances far beyond the stages supposedly characteristic of the homozygous yellow."

The main part of our stock is homozygous for recessive spotting, which occurs in black and brown, modified in about half of the cases by the "pink-eyed dilution factor." The factors which have a bearing on the data reported in this paper are:

D, dominant spotting; d, the allelomorph for dominant spotting.

P, dominant black eyes; p, the allelomorph which produces pink eyes.

DdPP and DdPp individuals are usually pure white with black eyes, but occasionally show some dark hairs on the rump and about the ears.

Ddpp mice are nearly always pure white but may have some light-colored hairs on the rump and about the ears. The pure white individuals resemble albinos, but microscopic examination of their retinae seems to show traces of pigment, and when mated together they produce some spotted young.

ddPp specimens have black eyes with spotted pelage; those with the formula ddpp have pink eyes with spotted pelage. The exact shade in the last two cases depends upon the presence or absence of P. The color of the eyes can be determined in early fetal stages.

My experience has been in accord with that of Little (7) and other students of dominant spotting in that I have been unable to obtain adult DD mice, but I noticed from the first that from Dd × Dd matings there were a certain number of aberrant offspring, a result corresponding with Detlefsen's findings.

In a total of 75 aberrant young born and observed in this laboratory, it has been found that these abnormal individuals consistently differ from the normal both in appearance and in length of life. Their skin is paler and contrasts strikingly with the deep pink color of the normal new born. When the blood of the abnormal young is compared with that of the normal from the same litter by means of a von Fleischl hemoglobinometer the readings are about 22.66 and 103, respectively. Because of the difficulty of obtaining sufficient blood for standard readings from an anemic individual, corresponding amounts of normal blood were used as a standard. Since the technique is identical, the figures form a reliable basis for comparison. Readings obtained with the Tallquist scale were essentially the same. Lange (6) in his work on the hemoglobin content of adult mouse blood obtained figures closely approximating these findings for the normal. The erythroplastids in the anemic blood averaged from 20 to 25 per cent. of the number found in blood from normal young in the same litter.

Intermediate phases were not present in any of the 75 cases observed, the true anemia being distinctive in appearance and therefore readily identified. That this anemia, although hereditary, is not comparable to the hereditary sickle-cell anemia of man described by Taliaferro and Huck (10) is indicated by the fact that the red blood corpuscles in the blood of the anemic mice do not assume elongated or crescentic forms in hanging drop preparations. Anatomically, the anemic differ from the normal young in having a smaller thymus and a larger heart. The spleens apparently show no hypertrophy, but the study of these and other organs is still under investigation.

The number of anemic individuals which appear in a litter is not constant, four out of seven being the highest number observed. No correlation was found between anemia and eye color or sex. The span of life usually varies from a few seconds to five days, although in one case an anemic mouse lived to be eight days old. At the time of birth they may be fully the size and weight of the normal young, but more frequently they are smaller. Despite the fact that they often ingest relatively large amounts of milk, each succeeding day finds them thinner and more emaciated until finally they die. We have not been able to prolong their life even by removing all normal young from the litter and leaving the adult female with only one or two anemics to nurse. The relative size of the normal and anemic young on successive days is shown in the figures. The litter of six, from which two normal mice were removed at birth, was from a Dd × Dd mating. The first photograph was taken two hours after birth, and the subsequent pictures at approximately 24-hour intervals.

The percentage of anemic individuals from different matings is indicated in Table I. The total percentage of

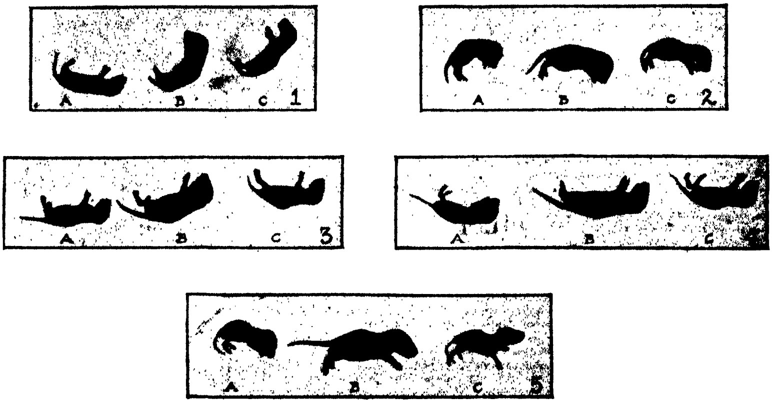

FIG. 1. Photographs of one normal and two anemic young from a Dd × Dd mating. All the pictures are made to the same scale; the first was taken two hours after birth, the following ones at successive 24-hour intervals. The three individuals are in the same relative position throughout, the normal control being in the middle. Both of the anemic young died before the end of the fifth day without increase in size. The usual amount of growth during this period is shown by the control.

TABLE I

OFFSPRING FROM DD × DD AND DD × DD MATINGS

Mating	Number of matings	Total young	Normal young	Anemic young	Percentage anemic	Average size of litter
Dd × Dd	122	500	425	75	15	4.098
dd × Dd	38	185	185	0	0	4.86

anemics was 15, but the frequency varied during the period of observation, being higher (17 per cent.) when the cages were inspected two or three times a day and lower (10 per cent.) when observations were taken once every 24 hours. This apparent variation was probably due to a differential death rate at or immediately following birth. Even the most frequent observations are not wholly accurate, for there is always the possibility of some young being eaten or lost before they can be observed. The average size of litters in both types of matings is shown in Table I. If the prenatal death rate is the same for both anemic and normal individuals, the expected size of the litters for the two groups should be identical, which condition closely approximates what was actually found. The insignificant discrepancy observed probably is due to the fact that some of the anemics were lost after birth.

To overcome the difficulties encountered in trying to tabulate accurately the young born, an attempt was made to determine whether anemics could be distinguished from normal fetuses in utero. The total gestation period of these mice is about 20 days, and by recording the time of mating the state of pregnancy can be ascertained. The pregnant females may then be opened and examined under an anesthetic and the condition of the fetuses observed. It was found that anemics are readily distinguishable from normals by the sixteenth day and that the living fetuses can be recognized as such both by their appearance and their movements. All the figures used in Table II are based on fetuses 16 days old or older.

Besides fetuses from parents both heterozygous for D, fetuses from dd × Dd matings were also examined. The results recorded for each group are indicated in Table II.

TABLE II

FETUSES FROM DD × DD AND DD × DD MATINGS

Mating	Total fetuses	Total living fetuses	Normal living fetuses	Anemic living fetuses	Dead implantations	Percentage of anemic among living fetuses	Percentage of dead implantations
Dd × Dd	270	211	159	52	59	24.64	21.85
dd × Dd	102	79	79	0	23	0.00	22.54

These figures show that in litters where there was the possibility of homozygous D genes anemics were present, but that in litters resulting from mating the same males to dd females, anemics failed to appear. The conclusion which this observation justifies is that the anemic young are those which are homozygous for D and that this anemia, which is hereditary, is due to homozygosis of the D gene or one closely linked with it.

Theoretically, the proportion of homozygous D's is 25 per cent., a proportion which corresponds almost precisely to the number of anemics found (24.64 ± 1.96). This close correspondence indicates that such fetal deaths as occur before the sixteenth day are uniformly distributed between anemics and normals and that the lethal effect of homozygous dominant spotting does not appear until the end of pregnancy. This is quite different from what has been previously supposed.

That the anemia is wholly a matter of heredity and not in any way dependent on defective diet seems to be clearly demonstrable. All the stock used was under one year of age. A certain number of both the Dd and dd groups were fed very carefully from the time of weaning, their food including milk, butter, cheese, lettuce, raw meat, sunflower seeds, peas, corn, wheat and mineral salts, but no difference was noted in either the number or the appearance of anemic young among litters of the Dd females when mated to Dd males. In many cases these Dd and dd females mated with the same males. The Dd females constantly produced some anemics, while the dd females produced only normals.

The anemic factor was also transferred to a strain in which both parents, brother and sister, had descended from a wild female and a Dd male. From the F_1 generation, in 13 uteri, 73 normal and 6 anemic fetuses were obtained. Among four litters born alive 29 normals and 6 anemics were found. The F_1 generation, back-crossed to dominant white mice, produced eight normals and one anemic.

Besides the normal and anemic fetuses in the uteri of dominant white mice, there are found embryos which have died after implantation. From Table II it will be seen that in Dd × Dd and dd × Dd matings the dead embryos represent, respectively, 21.8 and 22.5 per cent. of the total. Since the percentage of anemics found in utero is 24.6, a higher prenatal death-rate among this class could hardly account for the number of dead implantations, and since they occur in about equal frequency in both types of mating the possibility of their being associated with the homozygous lethal factor seems to be excluded.

Overcrowding has been suggested as an explanation for this form of prenatal mortality, but if this were the case one should find the number of dead fetuses far greater among the larger than among the smaller litters. From 112 pregnant uteri examined 50 contained litters of seven or over. Of these 50, 60 per cent. contained dead implantations, while from the remaining 62 uteri, including litters of six or less, 51 per cent. contained dead ova. Hence overcrowding does not seem to have been a very significant factor.

Similar fetuses which died early in gestation were reported by Ibsen and Steigleder (4). Some of these they identified as the missing homozygous yellows. There remains the possibility that many of the dead implantations in the uteri of dominant white and other mice represent a third lethal, the somatic appearance of which has not as yet been recognized. This is rendered the more probable, since in the inbred Dd and dd strains the incidence of this class approximates 25 per cent.

SUMMARY

The homozygous young of the dominant white mouse ordinarily live throughout the whole period of gestation and not infrequently for several days after birth. No evidence is found that their prenatal death-rate is greater than that of the normal individuals of the same litter. They are characterized, at least from the sixteenth day of gestation, by a special form of anemia which is the real cause of death.

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ACCRETION AND DISTENTION IN PLANT CELLS[1]

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GENERAL FEATURES OF GROWTH

THE fact that two distinct phases or stages, accretion and distention, may be recognized in the growth and development of the plant cell has been discussed in papers published during the last two years.

In the earlier accretion stage material is added to the protoplasmic mass, and changes in volume are due to imbibition. The young cell quickly passes into the distention stage, in which its volume is increased many times by the osmotic action of substances in solution in spaces, cavities or vacuoles in the protoplasm. The rate of increase reaches its maximum in this second stage; the distending pressures set up as a result of the relative permeability of the plasmatic layers and wall may be as much as 70 or 80 atmospheres, and great rigidity in such cell-masses is produced.

The actual amount of material or dry weight of the protoplasm shows no increase after distention or ballooning begins. On the contrary, it begins to shrink. This wasting of living material proceeds at the slowest rate in cells which show the least differentiation and remain in a parenchymatous condition. In highly differentiated tissues which serve mechanical purposes chiefly, the wasting or consumption of the protoplasm continues, so that wood-cells or tracheids, vessels, etc., soon reach a stage where only a trace of the reversible gels which make up protoplasm remain.

Measurements of growth which cover more than one cell would include the resultant rates of units in overlapping stages of accretion and distention.

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[1] Read by invitation before the American Society of Naturalists, Washington, D. C., January 1, 1925.

Increase in proportionate dry weight, as in animals, generally accompanies the development of the individual as in plants. The exceptions to this procedure are much more important among plants than among animals, and in many fruits, stems of succulents, sporophytes of the fleshy fungi and filamentous organisms the proportionate amount of water may be greatest at maturity.

THE INITIAL OF FUNDAMENTAL PROCESS UPON WHICH GROWTH RESTS

An attempt will be made on the present occasion to characterize somewhat more fully the physico-chemical processes which cause or characterize the two phases of growth of the cell. In so doing it seems hardly necessary for me to disclaim any idea that I may be presenting a completed scheme of metabolism, or a description of a continuous chain of events. I can scarcely do more than say that in the accretion stage, first protein and later carbohydrates are formed by condensation and synthesis and dehydration, and that in the second these substances are hydrolyzed and expanded. Some of these features have come in for discussion by several writers, but it may be profitable to make the picture as complete as possible at this time.

The union of molecules of two amino-acids in the formation of a protein would result in the formation of larger particles with a capacity for holding water less than the total of the separate molecules with a consequent dehydration. This would entail an accompanying or consequent setting free of water in the condensation or accretion stage of the protoplast. In such continuously acting cell-masses as the cambium the freed water would be taken up in the hydration of the older daughter cells which would be in a stage of expansion or distention by the accumulation of water in the syneretic spaces or vacuoles. Such a flow would in the case of the cambium be from protoplasts with a lower H ion concentration to a higher one.[2]

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[2] Pearsall, W. H., and Priestley, J. H. ''Meristematic tissues and protein isoelectric points.'' *The New Phytologist*, 22, 185. 1923.

The interlocking processes of accretion and distention are not, however, simply condensations and hydrolyses of proteins.

The activity of plant cells is notably characterized by the accumulation of carbohydrates as a constituent of the cell mechanism in greater proportion than in animals. It is true that combined carbohydrates occur in all nuclei, and that mucilages occur in the development of eggs, from the study of which the zoologist derives most of his cytological conclusions, but the condensation of sugars in the deposition of cellulose material in cell-plates and in wall-formation, as well as in the accumulation of the pentosans, mannosans, glucosans, pectins, etc., in the mass, is a procedure most noticeable in plant cells.

The hydrolyses of the proteins of the cytoplasm has been attributed to the action of basic proteins emanating from the nucleus by Spek. The hydrolysis of the cytoplasm in eggs is shown by Wilson[3] to follow the dispersion of the chondriosomes from their clustered position about the nucleus, who says:

> One might imagine, for instance, that during this association (*chondriosomes with nucleus*) the formed elements receive from the nucleus certain substances (enzymes or other chemical messengers) of which they become the carriers transporting them to regions of the cytosome where they play their specific part.

These speculative conclusions and disconnected observations are harmonized by my own observations that biocolloids consisting of proteins and pentosans show a coefficient of hydration in the presence of such amino-compounds as glycocoll, alanin, phenyl-alanin and histidin far in excess of that in water alone.[4] These compounds adsorbed by the chondriosomes would be carried toward the periphery of the cell until freed by meeting penetrating electrolytes.

The intrusion of the electrolytes also establishes new conditions of permeability in the protoplast. The plasmatic layer in which the proteins, pentosans and lipins are intermixed takes on a condition as a result of the interlocking and to some extent interfering action of the ions of K, Na, Mg, Ca and other bases as well as acid radicles in which it is variously permeable to these materials and all but impermeable to organic substances.

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[3] Wilson, E. B. "The Cell," 2nd ed., p. 346. 1925.

[4] MacDougal, D. T. "The distentive agencies in the growth of the cell." Proc. Soc. Exper. Biol. and Med., 19, 103. 1921.

Obviously a distending cell will reach its greatest turgidity when the complex plasmatic layer is most nearly impermeable to the dissolved substances in the cell, the activities of which result in the attraction of water. Such a condition of permeability has been thought by some writers to be coincident with an isoelectric condition of the proteins alone.

It seems to me so obvious that the permeability of a layer must depend upon the degree of hydration of its constituents that it does not appear profitable to elaborate the statement. Furthermore, differences in potential or the electric charges on the particles may originate in so many ways that I am not able to share the assumption that the activity of the hydrogen ion is the sole determining factor in the realm of physiological action. Its speed and penetrating action is greatest but in any mass of protoplasm its action may be controlled or masked by that of other carriers or electrolytes.

ISOELECTRIC ZONES

The gradients of hydrogen-ion concentration in the growing regions of stems are of the greatest interest. Thus the phellogen or embryonal layer which produces cork shows a high acidity denoted by Ph 3, equivalent to 0.001N HCl. Internally to it the cortex has an index of Ph 5.5 to 6.5. With regard to the vascular cambium, the phloem formed by it may be alkaline, Ph 7.8, and the xylem or woody cells as acid as Ph 5 or even Ph 4.3.[5] Among the more important implications are those which are concerned with the isoelectric points of the proteins. These substances would be condensed in the cells in which the hydrogen-ion concentration is identical with their isoelectric points, in which stage cells of protein alone would be least permeable. This is directly contrary to the statement by D. Haynes that permeability is at its maximum in a colloid in an isoelectric condition.[6]

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[5] See Pearsall and Priestley, as cited above.

Robbins has taken the maximum turgidities in cells to lie in the region of the isoelectric zones of proteins properly implying the condition of least hydration but in disregard of the effect of the presence of other material affected both by the hydrogen-ion concentration and the presence of electrolytes. A further series of observations of value in this connection was made by O. Arrhenius,[7] who found that the relation between the growth and Ph gave in most cases a curve with two maxima, so that a majority of plants seems to have two optimum hydrogen-ion concentrations—*e.g.*, *Pisum sativum* at Ph 6 and Ph 8, *Avena sativa* at Ph 5 and Ph 8, *Phleum pratense* at Ph 4 and Ph 9. Only one optimum is shown by *Lupinus luteus* (at Ph 6) and *Trifolium pratense* (at Ph 6).

The behavior of a protein cell is, however, a purely theoretical condition. The protoplast of the higher plant is a complex colloid, including a number of substances in the condition of a reversible gel.

In support of the contention that these points of maximum turgidity or greatest growth do not necessarily lie within the isoelectric zones of the proteins, I may cite results obtained by placing living cell-masses of Opuntia in various solutions and by tests of various kinds with constructed cells.

The succulent flattened stems of Opuntia are at all times in a pronounced acid condition. The total acidity of the sap may vary daily from an equivalent of 0.67 cc KOH 0.1 N to 1.48 cc per gram of dry weight. In the more acid condition distention is greatest in solutions at Ph 2.5: in the less acid condition the greatest distention is in solutions at Ph 3. On the other side of neutrality the greatest distention was in NaOH 0.0025 N — Ph 12, with no distinguishable difference which could be connected with the varying acidity. This is of especial interest in connection with the fact that a buffer situation exists by which the sap is maintained at about Ph 4. The higher maxima lies on the OH side of neutrality, an effect which might be due to the presence of Na and the formation of sodium proteinates.

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[6] Haynes, D. "The interpretation of electrical stimulation in terms of changes of hydrogen-ion concentration and the production of permeability in the plasma membrane." *Science Progress*, No. 70, pp. 223–233. 1923.

[7] D. M. in *Physiological Abstracts*, Vol. IX, No. 2, May, 1924. Arrhenius, O. Nagra bidrag til kannedomen om sambandet mellan markreaktionen och vissa kulturvaxters utoeckling. (*Meddelande No. 245 fran Centralanstalten pa jordbruksomradet*, Stockholm, 1923. 1–13.)

Such cell-masses placed in solutions of neutral NaCl showed the greatest distention in concentrations of 0.005M in the more highly acid condition; in the less acid condition the zone broadened so that no difference could be established in the expansion of the cell-masses in concentrations ranging from 0.0025 to 0.01M. Addition of such salts to the acid solution was followed by a maximum distention at PH 3 throughout the range of the material.

The addition of neutral salts to the hydroxide solution was followed by the greatest distention at PH 8 in the more acid condition and PH 11 in the less acid condition.

When these data are compared with the action of the constructed cells, which I have described in detail elsewhere, some interesting parallelisms are found.[8]

When only pentosans are included in the construction of experimental cells endosmotic action which would have produced distention in a protoplast was greatest in solutions at Ph 3, and in Na OH solutions at Ph 11. The inclusion of gelatine in such cells was followed by maximum effects at Ph 2.5 in acid solutions and Ph 11 to 12. The addition of lecithin to the materials of the cell was followed by broad zones of maximum action lying between Ph 2.5 to Ph 3 and in sodium hydroxide at Ph 12 (0.0025N).

[8] MacDougal, D. T. "The arrangement and action of material in the plasmatic layers and cell walls of plants." Proc. Amer. Phil. Soc., 63, 76. 1924.

If now we take into consideration the action of dead cell-masses which are nearly comparable to the constructed cells, the maximum action increased in acid solutions above Ph 2 and in hydroxides beyond Ph 11–12.

Such conditions of low permeability and resultant high distention and turgidity of cells have been interpreted by Robbins[9] as being determined by the isoelectric zones of the proteins. As such distention, however, depends upon the permeability of the plasmatic layer as a whole, and as this layer includes not only proteins, but pentosans and lipoids as well as intermediates, I have taken the ground that the maxima of distention in plant cells occurs under conditions in which all the substances in the complex plasmatic layer show a minimum permeability. For example, one of the two isoelectric zones of gelatine lies about Ph 4.7 and a cell constructed of this material should show greatest distention in an acid solution near this concentration. The constructed cell, including gelatine in its "plasmatic" layer, shows a maximum distention in acid at Ph 2.5 to Ph 3. Gelatine has a second isoelectric zone at about Ph 7.7. But the constructed cell including it has its maximum in the alkaline condition at Ph 11. These results are generally parallel to those obtained in dead cell-masses.

The parallelisms in the action of living cells, of dead cells and of constructed cells are conclusive evidence as to arrangement and general condition of material in the plasmatic layers and wall of the cell. The constructed cells display a pattern of action midway between that of living and dead cells, with respect to the H and OH and salt concentrations. Some of these differences may be ascribed to the buffer situation in the living cell, which is altered in dead cells, and has not been imitated in constructed cells. The complex set of ions of the common metals, which variously interfere and interlock, likewise can not be easily duplicated experimentally.

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[9] Robbins, W. J. "An isoelectric point for plant tissues and its significance." *Amer. Journal of Bot.*, 10, 412. 1923.

PERIODICITY

It is obvious that the period of development of an individual is a cycle, which in minute forms may be rounded out within an hour and in the great trees may stretch out over thirty or forty centuries. The periodicities or intermittent activities which are displayed by plants in regions with well-marked seasons are to be ascribed to alterations in the environment, or to variations in the food supply. Bloom and fruiting await the accumulation of material and seasonal variations in temperature, and are to be connected with no inherent property of protoplasm. Klebs has succeeded in flattening the seasonal curves of activity of some of our deciduous trees by cultivation under equable or uniform climatic conditions in greenhouses. It is commonly assumed that trees in tropical rain-forests show practically continuous growth, although this matter has never been subjected to critical examination. I have, however, made some records of the Monterey pine in the equable climate of the Coastal Laboratory at Carmel, California, of some importance in this connection. The behavior of one young tree in the habitat of the species may be cited in illustration preferably to a summarization of the activities of the large number under observation. These trees make new leaves on each year's extensions of the shoots which are connected with the wood of the previous year, and which persist, two, three or even four years. The tree is therefore evergreen and has normally at all times an adequate transpiratory and photosynthetic mechanism. The air temperature rarely drops to 0° C. and the cambium layer of the tree is usually at a temperature of about 9° to 11° C., at which growth proceeds at a high rate. The tree in question (No. 20) has a record growth from October, 1922, to January, 1925, in which there are no seasonal nodes. During a few hot dry days each summer the loss of water from the trunk masks the increases in the cambial region, but does not stop elongation. Winter storms late in January or in February are accompanied by low temperatures which stop enlargement of the shoots and trunk but not of the roots. The warm day following the coldest will be characterized by a rapid enlargement, and the tree will emerge from a drought shrinkage within five or six hours. Briefly, this pine may be characterized as one in which there is no inherent periodicity in the activities of the cork or vascular cambium.

The main conceptions presented in this paper are as follows:

SUMMARY

(1) Growth, or enlargement and differentiation, consists essentially of two groups of reactions, synthesis or condensations of proteins and carbohydrates in the accumulation of material in initial elements preceding and following mitosis, and subsequent hydrolysis and distention.

(2) The formation of proteins and of lipins characterizes the earlier stage. Beginning with the appearance of pentosans in the cell plate, condensation of the sugars progresses with a consequent increase of mucilages in the plasma and cellulose of the wall. Hydrolysis or hydration of the pentosan-protein plasma may be tentatively attributed to the action of acidic or basic amino-compounds which, emanating from the nucleus, may be carried into the cytoplasm by the chondriosomes. These substances would be freed from the chondriosomes by incoming electrolytes and are known to cause a greatly exaggerated swelling of biocolloidal mixtures simulating cytoplasm.

(3) Permeability, which is greatest in the youngest or undifferentiated stage, lessens toward maturity, probably by the action of absorbed and adsorbed electrolytes.

(4) The greatest distention of a cell takes place not precisely in the isoelectric zones of its proteins, but at a hydrogen-ion concentration and under other conditions of electrolytic action in which the complex layer is in a condition of least permeability.

able to take advantage of the age-long opportunity for the invasion of fresh waters, but demands an ability to cope with flowing water from the very beginning of the invasion of such a habitat.

It would not be difficult to imagine a course of evolution conditioned on a dynamic environment in the case of more or less elongated organisms firmly attached by one end of the body, but it is somewhat puzzling to picture any more or less freely moving organism being able to attain and remain in such a stimulating environment while these profound changes in body structure and form were taking place.

It is only just to state that such an inverted picture of evolution has never in so far as I know, been held by any competent ichthyologist. Nevertheless its enthusiastic approval by Grabau[2] in geological, and by Lull[3] in evolutionary texts since it was originally proposed by Chamberlin, seems to warrant an examination of its lack of basis in either fact or theory.

The typical form of the body in the fishes and in fact in other rapidly moving aquatic organisms is roughly fusiform, widest in front of the middle and slightly higher than wide. So common is this shape that Dean estimates that about half the existing fishes approximate this form. From it have probably been derived the dashing type as exemplified by the pickerel, the compressed type like the sunfish, and the depressed type like that of the angler or ray, all adapted for special habits of life.

The typical fish form, often called the stream-line form, does not imply that flowing water had anything to do with its origin. As a dynamical problem of perfecting a shape which would slip through the water with least resistance and without the production of eddies which are the chief factors in retarding progress, it is one that the fishes have solved even better than have the designers of torpedos and submarines.

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[2] Grabau, A. W., "Principles of Stratigraphy," Chapter 26, 1913.

[3] Lull, R. S., "Organic Evolution," pp. 462–465, 1917.

Nor are the fishes the only organisms that have perfected such a body form. Among invertebrates it is exemplified in the squids, which no one can doubt are of marine origin; in fact, much of their history is known and can be definitely correlated with their rapidity of movement through the medium and is not attributable to the movement of the medium. It must be obvious that any organism endowed with a forward motion brings about an environmental stimulus of exactly the kind which Professor Chamberlin can find only in streams. And that the former was the stimulus to metamerism as well as bodily form in the fishes and their ancestors it seems to me needs but to be stated to be accepted.

The squid propelled by an excurrent stream of water demands a stiffened axis, hence the retention of vestiges of the ancestral exoskeleton as the internal pen. A chordate like Amphioxus requires a flexible axis, hence the notochord. We are on safe ground in regarding the form and metamerism of the lancelet as giving a fair indication of the body form and habitat of the earliest fishes. That the various existing species of Amphioxus are rather sedentary in habit I regard as a protective adaptation and not as an indication that such a sedentary existence was the habit of the generalized ancestral stock of the vertebrates. Indeed, the metamerism of the lancelet proves that such was not the case.

This metamerism, which is a more fundamental part of the stream origin hypothesis than is the body form, and whose initiation and development is sought in the poetical simile of the waving of the submerged vegetation in a stream or the fluttering of a flag in the breeze, certainly preceded the beginnings of typical fish form. It is found in both marine worms and chordates, and it was unquestionably perfected in some of the chordates, and already present in slow moving eel-like forms, such as the lamprey. As Dean has shown, the simple metamerism of a type like the lamprey may readily be used as the starting point for deriving the more specialized metamerism which characterizes the different types of fishes as we know them to-day.

There are a number of collateral arguments that have been advanced, more or less tentatively, it is true, as tending to support the Chamberlin hypothesis. One of these is the peculiarity of the geological record of the earlier known fishes in that their fossil remains are infrequent except in certain deposits like the so-called passage beds between Silurian and Devonian, about which much difference of opinion has arisen as to the precise environment which they indicate.

Purely negative evidence is, of course, of little weight, but there are certain well-known considerations bearing on the absence as well as the presence of fishes in various parts of the geological column that have an interest in the present connection. The primitive fishes had a cartilaginous skeleton and the earlier Paleozoic fish-like vertebrates which figure so largely in the preserved record —the ostracoderms—are forms which developed an exoskeleton. It is this exoskeleton, or the dermal plates and spines of which it was composed, which constitute the bulk of the early records.

The ostracoderms are inconceivable as illustrations of the ancestral type of the true fishes and they are best regarded as early specializations adapted, more or less, to a bottom dwelling and grubbing existence. They certainly lack, for the most part, a body form adapted to swift movement or the capacity for maintaining themselves in currents of any velocity, except as bottom dwellers. The oldest known ostracoderms are those of the middle Ordovician and these occur in sediments of marine origin. Objection may be raised that stream dwellers frequently are carried, either alive or dead, to the sea margin and preserved as fossils in marine deposits, which consideration would perhaps conform to the sporadic occurrence of these forms in the earlier rocks.

That it really matters little in the present case is due to the fact that biologists are agreed that the ostracoderms as we know them in the rocks are highly specialized, that their origin must be projected backward to times long anterior to their appearance in the geological record, that they were derived from fish-like forms without durable parts and with presumably a more typical fish-like form, and that they are a sideline without issue and not in the direct line of ascent of the true fishes.

The other cul-de-sac of early vertebrate evolution, the Arthrodira, are much later to appear in the record, being confined to the Devonian and early Mississippian (Lower Carboniferous). Although their internal skeleton is sometimes superficially calcified, they owe their prominence in the geological record to the fact that, like the ostracoderms, they developed an armored exoskeleton. In the relatively abundant fish faunas of the Devonian, especially as exemplified in the Old Red Sandstone, now generally admitted as a series of predominantly continental deposits, we can see traces of adaptive departures from the normal fish form, showing that this was acquired long prior to the Devonian. For example, among the Dipnoi, *Dipterus* of the lower Devonian is much more fishlike than *Phaneropleuron* of the upper Devonian. The latter is more fishlike in body form than *Uronemus* of the Lower Carboniferous, and the last is much less retrogressive than the eel-like existing *Ceratodus* or *Lepidosiren,* as Dollo has pointed out.

In later geological times, with the more complete calcification of the skeleton, as exemplified by the so-called ganoid and teleost fishes we find an abundance of fishes in the rocks, so that we are forced to believe that the geological record is just what might be predicted if the earliest fishes were cartilaginous as all students of fishes believe. Only those with dermal armor are preserved in the earlier rocks, there being no trace of their cartilaginous ancestors or contemporaries. With the progressive calcification of the endoskeleton a relative abundance of fishes is found in the later marine sediments. With these considerations in mind I can not see that the sporadic occurrence of fishlike remains in the early Paleozoic has any bearing on the question at issue, for there might just as well have been millions of fishes in both land and marine waters in the early Ordovician and if they had no hard parts, both records would be equally barren.

A consideration of the fitness of the environment furnishes weighty *a priori* considerations for regarding the ocean as having furnished maximum facilities for the origin and evolution of life. Moreover, land waters are from the standpoint of geological time relatively evanescent features and for that reason can have played but a small part as theaters of evolution. The part of the earth's surface which at the present time supports the most abundant and varied population (if the geologically late Hexapoda be excepted) is the pelagic and sublittoral regions of the sea, and there is not the slightest reason to doubt that this has always been the case. The highly varied and swarming arthropod and brachiopod life of the Cambrian was, so far as we know, entirely marine. That so dominant a group of organisms as the trilobites did not invade terrestrial waters, if indeed they did not, for the evidence is of a negative sort and therefore not worth much, was due entirely, I believe, to dearth of food in fresh waters and not because they were dependent on a constant degree of salinity of the water.

In so far as we can judge from the sole survivor of the Meristomata (Limulus), or the existing Crustacea, they are to a very considerable degree oblivious to the degree of salinity of their environment so long as food is plentiful and the other environmental factors are not prohibitive. Many regard the primitive oceans as having been practically free from salts, and although there is considerable geophysical ground for such an opinion, such a condition was remotely anterior to that part of the past which is concerned with the origin of fishes, since the evidence of marine organisms with hard parts in the early Paleozoic and even in the pre-Paleozoic shows clearly that, if such was true of primordial ocean waters, that condition had long since been modified. Indeed, the evidence of the time involved and the extensive weathering of the pre-Cambrian rocks would in itself be sufficient proof of the probability of my statement.

The relative sparsity of organisms in the fresh waters of the globe and the variety of marine types which have never colonized these waters is one of the remarkable facts of biology. I regard the lack of permanence of fresh waters and their relatively poverty in food resources as a consideration entitled to great weight in any consideration of the place of origin of fishes or eurypterids. And if the fresh waters of the globe are to-day relatively poor in foodstuffs, how much poorer were they in the far distant Paleozoic before there were insects on the land or before the Mollusca or Crustacea or higher worms had invaded fresh waters. If the dominant early Paleozoic trilobites invaded the land as many students have supposed, it was probably by way of the strand, as did the Isopod Crustacea at a much later date, and not by way of fresh water. In fact, if there were no good arguments to be derived from other sources I would regard the foregoing considerations as amply nullifying the hypotheses that either the eurypterids or the fishes were fresh-water evolutionary products.

If the reader thinks of freshwater vegetation as a source of food it should be pointed out that we know casts of the alimentary canals of both trilobites and eurypterids and in both cases it is small and straight and indicative of a highly concentrated, non-vegetable diet.

Another possible argument has been based on the singular migratory habits of certain marine fishes, notably the various species of salmon and the shad, which have been shown by tagging experiments to return, often great distances, to spawn in the very stream in which they were hatched. Whether this is true of all fishes which enter streams to propagate is not known, but it has been suggested by Chamberlin and others that this might be considered as an indication of their return to the ancestral home of the race.

This homing instinct is also exhibited by the migratory birds and may be universally true of migratory animals, but that it means that the migratory birds originated in the region where they nest and rear their young is vigorously disputed by Brooks. It seems to me that it is about as logical and convincing as the explanation given by Crotch for the occasional migration of lemmings in Scandinavia, where those that survive the perils of the journey from the mountains across the lowlands are eventually drowned in the sea, which, Crotch states, they are endeavoring to cross to reach the lost Atlantis which their ancestors inhabited in Miocene times.

We are profoundly ignorant of the real explanation of this remarkable habit, but it may be remarked that it is one absent in many marine fishes and that many more go no farther than coming to the shore at the reproductive season, and at least one type—the eels—pass their mature existence in fresh waters and go far out to sea to spawn, possibly seeking for the rivers of the mythical Atlantis in which their ancestors first saw the light.

Moreover, lake fishes often show a similar habit in ascending tributary streams to spawn, and various lower marine organisms such as the squids and comatulid crinoids show a comparable instinct, coming from the depths into shallow water for reproductive purposes.

Brooks long ago suggested (*Pop. Sci. Monthly,* April, 1898) that migration in both fishes and birds might be considered a result of natural selection in the effort to find a safe place in which to lay eggs and rear young and that the enemies to eggs and young in the case of the fishes would be much fewer in streams than along the shoals of the seacoast. It certainly seems remarkable that such fishes as the shad or salmon, which are especially adapted for their marine habitat, and which apparently take no food during their freshwater journey, should be just the fishes that exhibit this homing instinct in its perfection.

One thinks of many factors, such as oxygen supply or hydrogen ion content or temperature, which might be correlated with the almost universal *wanderlust* preceding mating that might explain the habit. Meek,[4] who has published much on this subject, feels sure that the upstream tropism is due entirely to the current, and this may be the orientation factor, but it falls far short of explaining the preliminary sea voyage and coasting that precede the river trip.

Certainly unless one is prepared to admit that the salmon has a better racial memory than thousands of other marine fishes, one can not consider its habits as an argument in support of the hypothesis that the early vertebrates originated in streams.

SUMMARY

(1) The form and metamerism of fishes can only be explained as the result of the dynamics of a moving organism and not by the action of a moving medium on a sedentary organism.

(2) The latter could not maintain itself in a moving medium during the time required for its evolution into a form adapted for such an environment.

(3) The form and metamerism as exemplified in the earliest fishes were approximated long previous to the vertebrate stage, and were essential for the initial invasion of the streams by marine ancestors.

(4) The food content of the early streams was insufficient, and their duration in terms of geologic time was too transient, for them to have been centers of evolution.

(5) The geologic record of the fishes conforms exactly to the requirements of the argument, to wit: In the older rocks when the internal skeleton was cartilaginous there are no traces of fishes except a few sporadic occurrences of such as had developed a durable external skeleton: in the later marine rocks when the skeleton had become calcified there are abundant traces of fishes.

(6) The migration of a very small percentage of recent fishes from the oceans to streams for the purpose of spawning is shown to have no relevancy with regard to the original home of the race.

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[4] **Meek, A., Rept. Dove Marine Lab., No. 6, pp. 52–54, 1917.**

SHORTER ARTICLES AND DISCUSSION

CORRELATION AND MACHINE CALCULATION

AT various times during the past few months there have been rumors of wonderful new methods of determining correlation by the use of modern commercial machines permitting simultaneous multiplication and summation. The results now appear in a bulletin by Wallace and Snedecor.[1]

The need for quantitative work in biology is so urgent and the labor of adequate statistical analysis is so great that it may seem ungracious to imply any criticism of any discussion of methods of calculation which may be of use. There is, however, always a real danger that younger students, in accepting as wholly new methods which have long been known to biometrists, will fail to go to the original sources where the methods were first described and provided with illustrations of applicability.

About twenty years ago I recognized clearly the need for the development of formulae which would permit (*a*) the wider use of mechanical computing equipment, and (*b*) the organization of the routine work in such a manner as to attain the maximum number of end results and provide the maximum number of checks for the work with the minimum of arithmetical effort. The results have been given in a number of papers, the first three of which were published in this journal.

While I have assiduously practiced the scientific virtue of citing these papers of my own on every possible occasion, including both review[2] and criticism,[3] they do not seem to have come to the attention of the authors of "Correlation and Machine Calculation." That they have been used at all is perhaps largely due to the fact that I have personally taught at least a hundred biologists the advantage of one or more of these methods. In view of the fact that Wallace and Snedecor give only a very incomplete account of the whole problem of machine calculation, a brief reference to the literature may be helpful.

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[1] Wallace, H. A., and Snedecor, W., "Correlation and Machine Calculation," Off. Pub. Iowa St. Col. Agr. Mech. Arts, Vol. 23, No. 35, 47 pp. 1925.

[2] Harris, J. Arthur, "An outline of current progress in the theory of correlation and contingency," AMER. NAT. 50: 53–64, 1916.

[3] Harris, J. Arthur, "Reed on the coefficient of correlation," Quart. Pub. Amer. Stat. Ass. 15: 803–805, 1917.

The first and only essential for the use of modern computing and tabulating equipment in the rapid calculation of statistical constants is the calculation of the moments in terms of the actual measurements instead of in terms of deviations of these measurements from the true mean, or about some value selected arbitrarily but in a way to reduce arithmetical effort. As far as I am aware methods of doing this mechanically for the determination of the first several moments of frequency distributions were first given long ago by Hardy in the graduation of the British Office Life Tables. Hardy's method was subsequently described and elaborated by Elderton[4] (p. 19–23), and by Hardy himself.[5] It seems never to have been adopted in biological work, although it presents certain advantages when equipment adopted primarily for addition only is available. Hardy's method has the disadvantage that the moments are really taken about an arbitrary origin. This precludes the development of any general system of formulae.

In 1910 I pointed out the many advantages of taking the moments about 0 as origin. This is the essence of the method now described by Wallace and Snedecor for the determination of *r*. In this paper[6] the advantages of the method for raw and tabulated data, for the securing of checks on the accuracy of the arithmetical work, for the calculation of means of arrays for regression tests and for the calculation of the correlation ratio are fully set forth. Immediately afterwards essentially the same methods were applied to the formation of correlation and contingency tables[7] and of condensed correlation tables[8] in cases in which the number of possible combinations is large. These served as a basis for the development of a fairly complete system of formulae for the calculation of intra-class and inter-class coefficients from class moments under similar conditions.[9] This theory was the immediate basis for the suggestion of a substratum heterogeneity coefficient[10] which has been extensively applied in the analysis of agronomic experiments.[11] The possibility of spurious correlation due to disorderly differentiation in the use of such formulae was early recognized.[12]

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[4] Elderton, W. P., "Frequency-Curves and Correlations," London (1905).

[5] Hardy, G. F., "The theory of the construction of tables of mortality and of similar statistical tables in use by the actuary," London, 1909. See especially p. 59–63, 124–128.

[6] Harris, J. Arthur, "The arithmetic of the product moment method of calculating the coefficient of correlation," AMER. NAT. 44: 693–699, 1910. When this paper was written I was not aware that MacDonell had used (p. 231) the same formula for the product moment coefficient in a craniometric investigation published six years earlier. See W. R. Macdonnel, *Biometrika* 3: 231, 1904. His method, like that of Hardy, seems never to have been used by other biometricians.

[7] Harris, J. Arthur, "On the formation of correlation and contingency tables when the number of combinations is large," AMER. NAT., 45: 566–571, 1911.

[8] Harris, J. Arthur, "The formation of condensed correlation tables when the number of combinations is large," AMER. NAT., 46: 477–486, 1912.

Formulae for the calculation of the correlation between growth increments have been developed,[13] illustrated[14] and utilized in determining the probable error of the difference between two differences,[15] as illustrated elsewhere.[16]

Formulae for the correlation between a component, and between the sum of two or more components, and the sum of the remaining components of a variable have also been given[17] in terms of moments about 0 as origin.

The method of moments about 0 as origin was utilized in the development of a portion of the theory of correlation between a variable and the division of a dependent variable from its probable value, particularly in determining the standard deviation of the deviation of the dependent variable from its probable value and its regression on the independent variable.[18] It has also been used in determining the correlation between a variable and the deviation of an associated but not dependent variable from its probable value.[19]

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[9] Harris, J. Arthur, "On the calculation of intra-class and inter-class coefficients of correlation from class moments when the number of possible combinations is large," *Biometrika*, 9: 446–472, 1913.

[10] Harris, J. Arthur, "On a criterion of substratum homogeneity (or heterogeneity) in field experiments," AMER. NAT., 49: 430–454, 1915.

[11] Harris, J. Arthur, "Practical universality of field heterogeneity as a factor influencing plot yields," *Jour. Agr. Res.*, 19: 279–314, 1920.

[12] Harris, J. Arthur, "On spurious values of intra-class correlation coefficients arising from disorderly differentiation within the classes," *Biometrika*, 10: 412–416, 1914.

[13] Harris, J. Arthur, "Formulae for the determination of the correlations of size and of growth increments in the developing organisms," Proc. Soc. Exp. Biol. and Med., 18: 4–5, 1921.

[14] Harris, J. Arthur, and Reed, H. S., "Inter-periodic correlation in the analysis of growth," *Biol. Bull.*, 40: 243–258, 1921.

[15] Harris, J. Arthur, "The probable error of the difference between two differences," *Jour. Amer. Statis. Assoc.*, 18: 514, 1922.

[16] Harris, J. Arthur, Lawrence, Z. W., Hoffman, W. F., Lawrence, J. V., and Valentine, A. T., "The tissue fluids of Egyptian and Upland cotton and their F_1 hybrid," *Jour. Agr. Res.*, 27: 267–328, 1924.

[17] Harris, J. Arthur, "The correlation between a component, and between the sum of two or more components, and the sum of the remaining components of a variable," Quart. Pub. Amer. Statis. Assoc., 15: 854–859, 1917.

These papers owe their origin primarily to a realization of the need of replacing mental and manual work by purely mechanical methods. All give formulae and methods which may be useful to those interested in the surprisingly new idea of "correlation and machine calculation."

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VARIATION IN THE INTENSITY OF LINKAGE IN MAIZE

LINKED endosperm characters in maize provide excellent material for the study of problems of linkage, for each well-developed ear comprises a population of hundreds of individuals, and segregation ratios may be determined in the season in which pollinations are made. One known linkage group includes at least four genes affecting endosperm characters: *C c* and *I i*, which in the presence of certain other genes determine aleurone color; *Sh sh*, which affects the degree of shrinkage at maturity; and *Wx wx*, which affects the chemical composition. Studies of linkage in this group have been reported by Collins and Kempton[1] and by Hutchinson.[2]

Since the complementary action of *C* and *i i* is essential to the production of aleurone color, segregation for these two genes can not be studied in the same material. But by the use of appropriate material it is possible to determine simultaneously the linkage relations of *C c* (or *I i*), *Sh sh* and *Wx wx*. I am indebted to Dr. R. A. Emerson for supplying me with such material, which made possible the study here reported. This material was the seed of three ears, two of them heterozygous for *C c* and *Wx wx*, and the third heterozygous for *C c*, *Sh sh* and *Wx wx*, together with appropriate multiple recessive stocks for backcrossing. All the stocks are homozygous for *A A*, *R R* and *i i*, thus permitting the identification of *c c* by means of aleurone color.

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[18] Harris, J. Arthur, "Further illustrations of the applicability of a coefficient measuring the correlation between a variable and the deviation of a dependent variable from its probable value," *Genetics*, 3: 328–352, 1918.

[19] Harris, J. Arthur, "On the correlation between a variable and the deviation of an associated but not dependent variable from its probable value," *Jour. Amer. Statis. Assoc.*, 18: 393–394, 1922.

[1] IV. Conf. Int. Gen., pp. 347–356 (1911); AM. NAT., 46, pp. 569–590 (1912); U. S. Dept. Agr. Dept. Bul. 754 (1919).

[2] *Jour. Heredity*, 12, pp. 76–83 (1921); Cornell Agr. Expt. Sta. Mem. 60 (1922).

Significant differences in crossing-over in Drosophila, dependent on both environmental and genetic factors, have been shown in a number of recent investigations. Bridges[3] found that second brood progenies (from eggs laid during the second 10-day period) showed a significantly lower crossover percentage for certain genes of the second chromosome than did progenies of the first brood. Plough[4] obtained striking differences in crossover percentages in the second chromosome and in a portion of the third chromosome under different temperatures, the percentage of crossing-over being greatly increased by extreme temperatures both high and low. No difference in crossing-over resulted from temperature extremes in the remainder of the third chromosome or in the sex chromosome. The regions showing variation in crossover percentage under temperature differences coincided with those showing variation with age. Recently, Mavor[5] has been able to produce marked changes in crossover values by X-ray treatments, both in the sex chromosome and the second chromosome, crossover percentages being decreased in the sex chromosome and increased in the second chromosome by similar treatment.

Genetic differences in linkage intensity in Drosophila have been reported by Sturtevant[6] and Detlefsen.[7] Sturtevant found crossover percentages in the second chromosome greatly reduced [illegible] a strain of flies derived from a wild-type female from Nova Scotia. He demonstrated the existence of two genes affecting the amount of crossing-over in the second chromosome. Detlefsen was able by continued selection to reduce greatly the crossover percentage in the sex chromosome. In crosses between high crossover and low crossover flies thus produced, crossover values were more variable and consistently higher in the F_2 than in the F_1 generation.

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[3] *Jour. Expt. Zool.*, 19, pp. 1–21 (1915).

[4] *Jour. Expt. Zool.*, 24, pp. 147–209 (1917); 32, pp. 187–202 (1921).

[5] *Science*, 58, pp. 124–126 (1923); Proc. Soc. Expt. Biol. and Med., 20, pp. 335–338 (1923).

[6] Carnegie Inst., Washington, Publ. 278, pp. 305–341 (1919).

[7] Proc. Nat. Acad. Sci., 6, pp. 663–670 (1920); 9, pp. 149–156 (1923).

No direct investigations of germinal or environmental variation in linkage intensity in plants have been reported, to my knowledge, though a number of investigators have studied the relative crossover percentage in male and female gametogenesis. In discussing some apparently significant differences in crossover percentage in microsporogenesis and megasporogenesis in maize, Emerson and Hutchinson[8] point out that these may be due to disturbing conditions, such as temperature effects, rather than to inherent differences in the mechanism of crossing-over. Hutchinson[2] found crossing-over between *Sh* and *Wx* materially higher in his *I–Sh–Wx* stock than in his *C–Sh–Wx* stock, and suggested that possibly a crossover modifier such as that described by Sturtevant might be responsible.

The present investigation is planned as a study of genetic and environmental variation in crossing-over in maize. During 1923 the production of seed for future use was the chief concern, but it was possible incidentally to make a number of backcrosses for linkage data, using the heterozygous plants as seed parents. Data on crossing-over in the *C–Wx* region are available from 115 plants, including something over 50,000 grains. These data give some rather interesting indications which are the subject of this note.

The percentage of crossovers differed markedly in the three families, as indicated in the table below:

Family	Number of plants	Number of grains total	Number of grains crossovers	Crossover percentage
A	51	25,757	5,326	17.90 ± 0.15
B	20	8,308	1,879	22.62 ± 0.31
C	29	8,446	2,183	25.85 ± 0.32

The difference between families A and B is 4.72 ± 0.34, between families B and C 3.23 ± 0.45, and between families A and C 7.95 ± 0.35. All three differences are statistically significant according to any ordinary standard.

The crossover percentages and probable errors given above are computed in the usual manner from the total number of cross-overs and the total number of individuals in each group. They thus represent the results which would be obtained from determinations of the percentage of crossing-over between *C* and *Wx* in the three families independently. The probable errors take no account of the variation between the plants within the group —they are dependent only on the average crossover percentage and the total number of individuals (grains) within the group. Considering each family as a random sample from a population of plants varying in crossover percentage, the differences between the families might result simply from error of sampling. The data have therefore been reworked from this standpoint, the crossover percentages for individual plants in each family being averaged and the probable errors of their means determined by Bessel's formula. The results are as follows:

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[8] *Genetics*, 6, pp. 417–432 (1921).

Family	Mean crossover percentage
A	18.14 ± 0.26
B	22.47 ± 0.47
C	25.99 ± 0.43

The differences are as follows: between A and B 4.33 ± 0.54 per cent., between B and C 3.52 ± 0.64 per cent., between A and C 7.85 ± 0.50 per cent. Although the probable errors have been increased in all cases, all the differences remain clearly significant.

It is of course possible that different environmental conditions during the maturation period may account for these differences. The flowering dates, which were noted for each plant, averaged about two days later in family B than in family A, and about three days later in family C than in family B. But fifteen plants of family A, from a planting two weeks later (flowering dates about 8 days late) gave an average crossover percentage of 20.11 ± 0.68, which is not significantly different from that of the first planting of family A, as given above. Moreover, no relation of flowering dates to crossover percentages was apparent within any of the families.

Environmental variation in linkage was indicated by the relatively wide differences in crossover percentage determined from different ears of the same plant. If these differences are due only to error of sampling differences greater than their probable errors should occur in only about one half of the cases, and the expected frequency of differences of various multiples of the probable error can easily be determined. Comparisons of crossover percentages of different ears of the same plant may be made in 47 cases in family A (first planting).

The occurrence of differences of various extent in comparison with their probable errors, together with the number expected if the differences were due entirely to error of sampling, are shown in the following table:

$\frac{\text{Difference}}{\text{P.E.}}$	Probable occurrence in 47 trials through error of sampling	Observed occurrence
1.0 or more	23.5	29
1.5 or more	14.7	24
2.0 or more	8.3	18
2.5 or more	4.3	11
3.0 or more	2.0	7
3.5 or more	0.9	5
4.0 or more	0.3	2
4.5 or more	0.1	1

The rather wide differences in crossing-over between ears of the same plant suggest that significant differences in crossover percentage in megasporogenesis and microsporogenesis, such as those reported by Emerson and Hutchinson, may be due merely to different environmental conditions during the maturation of male and female gametes, a possibility which was suggested by those authors.

Of the 33 plants in family A in which the crossover percentage for the first and second ear was determined, 20 gave a higher percentage in the first ear, but the average difference was less than 1 per cent. and was not statistically significant.

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THE HOST-PARASITE METHOD AND THE DISTRIBUTION OF FROGS

IN the March-April number of the AMERICAN NATURALIST for 1923 I attempted to apply the well-known dispersal theses of Matthew to the Amphibians. In dealing with the *Salientia* I came to no new or striking conclusions, as I utilized the recent classificatory work of my friend Noble, and my notions of their dispersal were quite in accord with his.

Metcalf, in his work on the *Opalinidae,* came to quite different conclusions regarding the dispersal of their hosts, and has recently reiterated these conclusions in order to refute my statements.

Many elements enter into the discussion of this matter, and conclusions as to dispersal are the final results drawn from consideration of certain facts and from the interpretation of those facts.

The first set of facts to be considered is the whole mass of data regarding the anatomy, phylogeny, distribution and classification of the *Opalinidae.* These facts have been discovered and set forth by Metcalf and I find myself perfectly willing to accept them at their face value.

The second set of facts to be considered is the similar data regarding the anatomy, phylogeny, distribution and classification of the *Salientia.* Here, of course, the matter becomes herpetological, and many of Metcalf's statements are open to attack.

Third, there is the question as to whether these two sets of facts coincide, for upon this coincidence of the classification and distribution of the two groups hangs the right of the parasitologist to discuss anything regarding the hosts.

Fourth, even if this coincidence is found to exist, there remains the question as to its meaning and its applicability to discussions of dispersal.

Regarding the first set of facts, I have nothing to say. Regarding the second I find many statements by Metcalf with which I am compelled to disagree. I hasten to admit that most of these herpetological pronunciamentos to which I object have no bearing on the argument, as, for instance, *Liopelma,* the New Zealand frog, which is, according to Metcalf, a Leptodactylid. This frog is absolutely known to be a Discoglossid, yet, as its parasites are unknown, there is no reason for its inclusion in the present discussion.

The recognition of the *Brachycephalidae* as a natural family, which Metcalf denies, would really strengthen his case, for their parasites are not those of the *Ranidae,* with which they are usually associated in the classifications, but rather the parasites of the *"Leptodactylidae"* from which, according to Noble, the *Brachycephalidae* have arisen.

Still more remarkable and more pertinent is his statement that the families *Ranidae* and *Hylidae* do not compete because of their different habits, and that therefore there is no reason for the absence of *Hyla* in Africa and in the Oriental region. This statement is made in the face of the well-known and elaborate arboreal adaptations of Ranids in these very regions, facts which have often been used to explain the absence of Hylids.

More to the point, however, is the question of the coincidence of data drawn from both host and parasite. 38 genera and 163 species of frogs have been found parasitized by *Opalinidae*, and upon this rather slender sample of the Salientian fauna of the world hangs Metcalf's whole scheme of Salientian dispersal. There is little specific infection noticeable. 41 of the 163 species of frogs are known to harbor more than one species of parasite, and 14 species of Opalinids infect more than one host. Nor is the case much better when we examine genera rather than species. Genera are *not* marked by uniformity of parasite. In only five of the 38 genera have five or more species been found parasitized. These are *Bufo*, *Hyla*, *Rana*, *Scaphiopus* and *Leptodactylus*. There are four genera of *Opalinidae*. *All four* of these infect each of the genera *Bufo*, *Hyla*, *Rana* and *Scaphiopus*. *Leptodactylus* alone of the genera in which five or more species have been found parasitized is infected by a single genus of parasite, *Zelleriella*.

When one inquires into this situation, so unfortunate for the host-parasite method, one is forced to surmise that it is barely possible that the parasites themselves have their own distribution which does not altogether correspond with the distribution of their hosts. Thus, in the genus *Hyla*, so far as known from the 23 species found parasitized, the Hylas of Australia have *Protoopalina*, in North America and in Eurasia they have *Opalina*, whereas in South America they have *Cepedea* and *Zelleriella*, with an occasional *Opalina*. Here is a complete lack of that coincidence of the classification and distribution of the two groups upon which hangs the claim of the parasitologist to discuss the dispersal of the host. And the distribution of the genus *Hyla* is one of the great puzzles of herpetology which the host-parasite method is so suited to solve!

Even if this coincidence is proven to exist, and it seems somewhat sadly to seek, it has been taken by Metcalf for much more than its face value. I can not regard the host-parasite method with the proper awe. It is a valuable check and nothing more. And it is a check on the *classification* of the hosts, not on their dispersal. We have to explain discontinuous distribution of the hosts. In any such case there are two possibilities: either the classification of the hosts is erroneous and the two components form an unnatural assemblage of forms, or the classification of the hosts is correct and the two components form a natural assemblage of forms and a once continuous distribution has become discontinuous. The host parasite method may help us to decide which of these two propositions is true. Beyond this it is not only valueless but misleading, for if the classification of host and parasite agree our hearts are a bit more strongly inclined to believe that we are dealing with a natural group whose distribution has become discontinuous, and we are where we started, even though somewhat more firmly fixed in our original position. *Any* theory of dispersal which will enable the host to arrive at its present estate will obviously serve equally well for the parasite which accompanies the host on its peregrinations.

Let us now pray humbly that light may be vouchsafed us and examine two of the outstanding anomalies in frog distribution upon which we have the new parasite data.

The first of these is the presence of *Hyla* in Australia and in South America in numbers, its relative scantiness in the Holarctic region, and its rarity or non-existence in Africa and in Indo-Malaysia.

The Hylas of the northern world have *Opalina,* the Australians *Protoopalina,* and the South Americans have *Cepedea* and *Zelleriella,* with occasionally *Opalina.* Here there is little to enlighten us. Additional evidence that the Eurasiatic Hylas are related to North American forms. *Perhaps* additional evidence that Southern Hylas are more primitive than Northern, since *Opalina* is the most specialized genus. No evidence from parasitology that Australian and South American Hylids are closely allied. No *additional* evidence from parasitology as to the route by which the *Hylidae* have reached their present homes.

It would be very interesting to know the parasite of the Abyssinian *Hyla wachei,* which is apparently allied to South American forms.

The second anomaly is the presence of toothed Bufonids (Leptodactylids) in South and Central America and in Australia and their absence from the rest of the world. The toothed Bufonid of South Africa, *Heleophryne,* is not admitted to the discussion, as its parasites are unknown. The facts in the present instance are that the toothed Bufonids of Australia and South America are both infected by *Zelleriella* and by *Protoopalina*. *Zelleriella* is found only in Australia and in the New World. Here is indeed that happy coincidence of the classification and distribution of the two groups for which we sought in vain in the case of the similarly distributed *Hylidae*. Here is the prize exhibit, and what does it prove? It is *additional* evidence that the toothed Bufonids are a natural group. No evidence whatever as to the routes whereby these Zelleriella-bitten Bufonids arrived in Brazil and Botany Bay. We are left as much in the dark as to the travels of Salientia as we were before the advent of this *lucus a non lucendo*. And this does not even take into account the fact that arboreal Ranids may easily be the cause of the general disappearance of Hylids from Africa and from Indo-Malaysia; or that the single *Hyla* and single-toothed Bufonid in Africa require a whole additional set of coincidental infections to explain them rather than the general excommunication which Metcalf has pronounced against them because their parasites are unknown. Indeed, these two African cases go far to render the triradial northern dispersal the more attractive theory and incline our hearts more and more to relegate the whole Bifrostean hypothesis to the domain of mythology, whence came Atlantis and St. Brandan's Isle and Tir-nan-oge.

These two examples of Hylids and toothed Bufonids are in no way explained by the additional data derived from their Opalinid parasites. And yet these two cases are the cornerstone of the whole edifice, the keystone of the landbridge so airily cast across the longest east-west stretch of water in the world, the South Pacific Ocean. To this bridge the Opalinids offer no real support. Upon what then are they based? It is the old, old story of the similar fauna of South America and Australia. The old bridge originally erected for the marsupials and fallen down upon the discovery of fossils in the North is now hoisted once more from the Globigerina ooze and serves to transport the poor parasitized Leptodactylids, which were not the cowanderers of the marsupials, because, forsooth, no fossils are known.

The actual argument, then, is as follows:

Marsupials and Leptodactylids have similar distributions.

Marsupials came from the north because we have fossils.

Leptodactylids, on the contrary, came from the south, because we have no northern fossils.

Thus are similar ends attained by different methods, because small creatures like frogs do not make (1) many (2) identifiable fossils.

A final word on "the geographic center of the group range," which is "the center of available migration routes," and which I proposed as perhaps the best method of determining the center of dispersal of a group, inasmuch as the location of the primitive members or the specialized members is not necessarily the center, as Matthew and myself have shown.

This geographic center is eschewed by Metcalf as geometric rather than biologic. Yet it seems to be used by him with less salt than I should have judged possible from the Petrine fervor of his denial; for nothing can be more neatly geometric than the straight and narrow path, built upon the abyssal ooze and connecting South America and Australia; although the more biological qualification, "the center of available migration routes," seems to suffer from neglect.

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FORTUITOUS VARIATION

THE theory of natural selection takes for granted the occurrence of heritable variations in all parts of all organisms. Its effectiveness as a working principle depends on the number and extent of such changes. Observations show that variations are universal in organisms and that they are fortuitous; that is, occur according to chance. According to this view there is no more reason to expect variations to appear in one direction than in another, or that there should be more favorable variations than unfavorable.

All variations, however, can not be classed as favorable or unfavorable. Some occupy a neutral position, neither stimulating the growth and reproduction of the organism nor inhibiting its development and propagation. Such variations do not come under the influence of natural selection, because they are of no selective or survival value. It is of little consequence to the organism whether these variations persist or not, as the organism can live and reproduce as well with them as without them. Color of grain in corn and slight heritable increases or decreases in oil or protein in seeds are examples.

Darwin stated that our ignorance of the causes of variation is profound. This describes the situation as well to-day as it did at the time this statement was made. Lamarck believed that the direct action of environmental conditions was responsible for the appearance of heritable variations, but this has not been proved. Various attempts have been made to induce mutations by artificial means. To date, however, with one or two possible exceptions, the results have been negative. Only a few years ago there were supporters for the idea that selection, carried on in a definite direction over a period of years, induced heritable changes in that direction. Such changes have now been interpreted in other ways. Furthermore, the character is the manifestation of the factor which precedes the character in ontogeny, and the character must appear, of course, before selection can become operative. Hence, selection can scarcely be said to induce germinal changes when such changes had already taken place in the germ plasm before the organism was subjected to selection.

Whatever may be the cause of variations, it appears certain that they are not induced by a need for them on the part of the organism concerned. In favor of this view is the idea above expressed; namely, that variations occur in characters that are neither favorable nor unfavorable or in degrees that are so slight as to preclude the possibility of their being of any selective or survival value to the organism. Furthermore, there are cases in which variations are known to have occurred prior to the time they are needed to preserve the organism, and hence are already present when needed. This is exemplified in variations enabling the plant to resist fungus diseases and insect pests.

The above comments are suggested by results recently obtained in an experiment on breeding soybeans containing either high or low oil content at the Illinois Agricultural Experiment Station. In 1922 several hundred plants were analyzed separately for oil content. Considerable variation was found. No doubt some of this variation was due to environmental factors, though all plants were grown under as nearly the same soil and climatic conditions as possible. That some of this variation was genetic, however, is indicated by progeny tests. To determine to what extent the extreme variations in oil percentage were inherited a number of the highest and the lowest plants in oil content were selected and progenies grown in 1923. The results are shown in Table I. All the progenies but one from the high parents were very similar in oil content. The exception (Progeny No. 2021) is significantly lower than the rest, even though its parent gave approximately the same analysis as the other high selections. This is but another instance of the effect of the environment in masking the genetic constitution of an organism. Progenies of the low selections were much less consistent in oil content than those of the high selections. Many of them showed significant differences when compared either with one another or with progenies from the high selections. This is a strong indication

TABLE I

PERCENTAGE OF OIL IN PARENT PLANTS AND PROGENY

	Parent No.	Analysis of Parent	Progeny No.	Mean per cent. oil of progeny	Difference	$\frac{\text{Difference}}{\text{Probable error of diff.}}$
High Selections	1590–5	21.23	2018	18.66 ± .0832		
	1590–6	21.52	2019	19.15 ± .1176	1.30 ± .2580	5.04
	1590–10	21.35	2021	17.85 ± .2296		
				15.29 ± .0915		
	1590–16	21.56	2023	18.97 ± .0814		
	1590–17	21.78	2024	19.20 ± .0941		
	1590–19	22.59	2025	19.57 ± .0779		
	1590–22	21.54	2026	18.62 ± .0798		
	1590–28	21.53	2027	19.33 ± .1037		
	1590–35	21.96	2028	19.86 ± .1136		
	1590–43	21.07	2029	19.97 ± .1387		
	1590–44	21.56	2030	19.96 ± .0912		
	1590–57	21.20	2032	19.61 ± .0977		
	1590–62	21.84	2033	20.10 ± .1114		
	1590–65	21.75	2034	19.50 ± .1769		
	1590–69	21.88	2036	19.74 ± .1175		
	1590–77	21.98	2038	20.18 ± .1135		
	1590–84	21.57	2039	20.20 ± .0997		
	1590–89	21.82	2040	19.54 ± .1508		
	1590–91	21.79	2041	19.19 ± .0819		
	1590–92	21.77	2042	19.58 ± .0862		
	1590–136	21.82	2045	20.35 ± .0859		
	1590–171	21.79	2047	19.89 ± .1058		
	1590–175	21.72	2049	20.60 ± .1089		
	1590–191	21.62	2050	20.19 ± .1383		
	1590–197	21.67	2052	18.99 ± .1160		
	1590–204	21.68	2053	20.15 ± .0764		
Low Selections	1590–8	18.33	2020	15.29 ± .0915	2.20 ± .1476	14.91
	1590–67	18.38	2035	17.49 ± .1158		
	1590–72A	18.16	2037	18.10 ± .0787	2.22 ± .1288	17.24
	1590–96	17.99	2043	15.88 ± .1020		
	1590–162	17.82	2046	19.70 ± .0888	3.30 ± .2670	12.36
	1590–172	17.96	2048	16.40 ± .2518		
	1590–195	18.09	2051	17.00 ± .1194		

that some of the variations shown by the parent plants grown in 1922 were heritable. Hence, in this variety, at various times in the past, germinal changes have occurred affecting oil content, and as a result the variety is a mixture of types so far as this character is concerned. The heterogeneous nature of the variety with respect to oil content would never have been detected had analyses not been made.

Similar statements can be made respecting resistance to plant diseases. Heritable variations occur from time to time, irrespective of whether the variety is ever subjected to attacks of the causal organisms or not. As a result, when such attacks occur, individual plants are found which are partially or wholly resistant and hence are able to survive and propagate themselves. In this way resistant varieties are produced. Resistance to plant disease, therefore, is not a variation induced by a need for it, felt by the individual plant; for such a variation occurred prior to such need. *Also, if, after the attacks of the causal organism, further variations occur in the direction of more nearly complete resistance, it seems reasonable to suppose that the same cause or causes which produced the initial variations are operating to bring about further germinal changes, rather than that the result is due to the presence of the causal organism.*

Variations of the type mentioned are physiological rather than morphological. In a few cases disease resistance in plants may be concerned with form or structure, but it is probably, in most cases, bound up with the physiological processes of the plant. Physiological characters are of great importance to the plant in growth and reproduction. The difficulty is that these characters can usually be detected only by performance or behavior rather than by appearance. Heritable variations in such characters are of considerable importance in plant breeding, but such variations are hard to study and fix. Winter hardiness, resistance to lodging, ability to endure keen competition and efficiency of root systems are typical examples of characters which are considered physiological in nature.

It is deemed safe to predict that future work in plant breeding will be concerned more and more with physiological characters. Progress along this line is not hindered by the lack of variations, but rather by inability to detect them. As new and more refined methods are developed it will be possible to isolate lines which excel in this or that physiological character and by combining these lines through hybridization, to produce types better adapted to specific purposes than those existing at present.

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FERTILITY AND TOLERATION OF TEMPERATURE IN INBRED DROSOPHILA[1]

THE amount of variability with regard to the toleration of temperatures above the normal range exhibited by different wild strains of *Drosophila melanogaster*, reported in a previous paper,[2] suggested the possibility of segregating lines by inbreeding within a single strain which might show similar variations.

With this possibility in mind, a single pair of flies was segregated in January, 1923, from a mass culture of Drosophila collected in Sweden by Dr. G. Bonnier, and three pairs of the first generation offspring were used to start three lines of brother-sister matings. This strain of Drosophila had been kept in the laboratory for the preceding three months and had shown high viability in connection with other experiments. The three lines were kept at room temperature in quarter-pint milk bottles containing the usual banana agar culture medium which has been found to give fairly uniform conditions. The Sweden stock was run simultaneously in mass cultures.

In the fifth inbred generation the "C" line developed an eye mutation[3] which was carried in the line during the experiments. With this exception the four sets ran uniformly, varying only slightly in time of hatching and development until August, when the three lines were in the fourteenth inbred generation. At the time of hatching out the fifteenth generation the lines were at Woods Hole, where they were subjected to considerable temperature fluctuation from the heat of August days to the cool nights.

[1] From the Biological Laboratory of Amherst College, Amherst.

[2] Plough, H. H., and M. B. Strauss, *J. Gen. Physiol.*, 1923–24, vi, 167.

[3] This mutation was found to be identical with one, designated as Brown-II, which has been isolated from the Columbia University cultures of the Sweden stock. It is an allelomorph of the Brown described by G. Waaler, *Hereditas*, 1921, ii, 391, located at 105, in the second chromosome. Brown-II is quite similar to Purple, and the double recessive is indistinguishable from either Brown-II or Purple. In older flies, whereas Purple seems to fade toward the wild eye color, Brown-II is accentuated.

The "C" line produced its offspring at the usual time, but it had progressed another generation before a fertile pair could be secured from the "A" line, and both of these developed one generation further before the "B" line yielded a pair of fertile progeny. That the climatic changes caused an effect on the germ plasm at this point seems less likely than that there already was a genetic difference between the lines as a result of the inbreeding which manifested itself for the first time under the less favorable environmental conditions.

TABLE I

Number of Offspring Hatching from Pair Matings in the United States

Date of counts	Line and generation inbred	Bottle number			Average number of progeny per fertile bottle
		1	2	3	
1923					
Oct. 15–25	A–17	86	67	16	56
	B–16	34	21	13	23
	C–18	212	208	183	201
Oct. 28–Nov. 6	A–18	80	7	2	30
	B–17	10	4	*	7
	C–19	184	183	177	181
Nov. 8–18	A–19	138	114	48	100
	B–18	63	37	34	45
	C–20	227	209	199	212
Nov. 18–28	A–20	57	16	*	36
	B–19	23	10	6	13
	C–21	213	211	177	200
Nov. 29–Dec. 8	A–21	204	39	*	121
	B–20	8	7	6	7
	C–22	256	213	208	226
Dec. 13–23	A–22	177	120	69	122
	B–21	30	7	6	14
	C–23	305	262	254	273
1924					
Jan. 30–Feb. 7	A–26	70	*	*	70
	B–25	17	6	2	8
	C–27	199	187	181	189
Feb. 10–20	A–27	223	147	89	153
	B–26	37	8	*	22
	C–28	237	227	203	222
Summary of	A	total	progeny	1769	88.5
eight gener.	B	"	"	389	17.6
	C	"	"	5115	213.1

Upon the return of the cultures to Amherst they were kept in an electrically controlled incubator at 24° C. in order to eliminate as far as possible environmental changes during the tests. The first determinations made were on fertility as measured by the number of progeny hatching from each pair mating in ten days. The progeny of three pairs of flies were counted in each of the inbred lines. In a set of counts made on eight different generations, spread over five months, and involving over 7,000 flies, it was found that the "C" line gave an average of 213.1 flies per mating, the "A" line 88.5, and the "B" line 17.6. The results of the individual counts are summarized in Table I. The fact that the "C" line, carrying the eye-mutant Brown-II, developed many more imagos than either of the other two lines is interesting in connection with the work of Gonzalez,[4] which indicated that the eye-mutant Purple is connected with a large number of offspring.

A further test of the differences between the lines was offered by egg counts. The method of making the counts was one used by Plough, to whom I may at this point express my indebtedness for his suggestions in all the work. The ordinary banana agar was poured in a thin layer on microscopic slides for about half their length and allowed to set with a plate of glass resting on the agar, thereby smoothing the surface. These were placed in the usual fly bottles with a piece of toweling to absorb moisture in the bottom and the flies introduced. The slides were changed daily, and the eggs laid on the agar counted under a binocular microscope. Following this, the slides were placed in a bottle containing food and allowed to hatch.

The "C" line laid the most eggs, five times as many as the "A" line and six times the "B" line. Although the two latter lines were not very different in the number of eggs laid, 70 per cent. of the "A" line eggs developed into imagos, while only 25 per cent. of the "B" line did so. The "C" line gave about 40 per cent. development, the smallness being possibly accounted for by the crowding of larvae on the slide before they were large enough to reach the other food. The counts are shown in Table II.

Since these results suggest that the difference in numbers of offspring between the lines depends on the number of eggs laid,

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[4] Gonzalez, B. M., Amer. Natur., 1923, lvii, 289.

TABLE II

EGGS LAID IN PAIR MATINGS AND IMAGOS HATCHING IN INBRED LINES

Line and generation	Eggs in ten days from bottle number		Total eggs	Total imagos hatching	Percentage development
	1	2			
A–23	113	18	131	93	70.9%
B–22	59	46	105	27	25.7%
C–24	324	299	623	242	38.8%

one would expect that in first generation crosses the number of progeny would depend on the female line. Castle in his work had found similar results with crosses between lines.[5] To check this up crosses were made between the three lines reciprocally, the results being summarized in Table III. In all six crosses the number of offspring is seen to be approximately the same as for the pure line from which the females were secured. The slight favorable effect of using foreign males, though hardly large enough to be significant, is in line with Hyde's results.[6] However, in the second generation of the crosses shown in Table IV, the average progeny per pair was considerably higher than any

TABLE III

NUMBER OF PROGENY PER PAIR MATING IN FIRST GENERATION CROSSES

Line and generation ♀ × ♂	Bottle number			Average number of progeny per fertile bottle
	1	2		
A–25 × B–24	134	112		123
A–25 × C–26	182	2		92
B–24 × C–26	27	3		15
B–24 × A–25	*	*		*
C–26 × A–25	225	225		225
C–26 × B–24	220	201		210
	1	2	3	
A–25 × A–25	70	*	*	70
B–24 × B–24	17	6	2	8
C–26 × C–26	199	187	181	189

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[5] Castle *et al.*, *Proc.* Am. Acad. Arts and Sci., 1906, xli, 731.
[6] Hyde, R. R., *J. Exp. Zool.*, 1914, xvii, 173.

of the pure line averages, similar to the results of both Castle and Hyde (*loc. cit.*). This suggests that the factors which had depressed the number of offspring in the pure lines were dissimilar and thus the restoration of the heterozygotic state brought the totals beyond those of any of the pure lines.

TABLE IV

PROGENY IN SECOND GENERATION OF CROSSES

Grandparents ♀ × ♂	Bottle number			Average number of progeny per fertile pair
	1	2	3	
A–25 × B–24	293	221	213	242
A–25 × C–26	349	339	271	319
B–24 × C–26	367	321	252	313
B–24 × A–25	no F_1			
C–26 × A–25[7]	·			
C–26 × B–24	330	289	249	289
A–25 × A–25	223	147	89	153
B–24 × B–24	37	8	*	22
C–26 × C–26	237	227	203	222

During the same period that the control temperature counts were being carried on, tests were made of the relative ability of the lines to tolerate high temperatures. Four sets of tests, involving at least three bottles per line in each test, at temperatures ranging from 30° C. to 32.5° C. showed the three inbred lines unable to produce any second generation offspring, with the exception of a single bottle in the "C" line which just gave enough offspring to carry the line for five generations, one bottle a generation, before being discontinued. In the last test, at 30° C., the Sweden stock and the crossed lines were also tested, but likewise failed after the first generation.

A fifth test, however, made at 29.5° C., produced second generation offspring in the "C" line, the three crossed lines, and the Sweden stock. Two of the crossed lines and the Sweden stock were further able to produce a third generation. This indicates that the same type of genetic differences existed in these lines, all descended from the Sweden strain, as had been noted to exist between different strains in the former work. In the first generation hatching at this temperature, the numbers of offspring, shown in Table V, though not as large, bear the same relation to each other as in the control tests.

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[7] An accident prevented any counts being made, but the reciprocal cross of this as well as the reciprocal cross in the case where no first generation hatched is illustrative.

TABLE V

DIFFERENCE IN TOLERATION OF TEMPERATURE OF 29.5° C.

Line and generation	Bottle number		Average number per fertile bottle	F_2	F_3
	1	2			
A–29	65	20	42	failed	
B–28	6	*	6	failed	
C–30	74	67	70	bred	failed
Sweden	88	81	84	bred	bred
AB cross	132	111	121	bred	bred
BC cross	173	121	147	bred	bred
AC cross	147	30	88	bred	failed

SUMMARY

(1) By inbreeding for from fifteen to thirty generations, three lines of Drosophila were secured from an original pair of wild stock flies which showed clear differences in fertility as measured by offspring produced.

(2) The numbers of eggs laid by females of each of these lines showed similar differentiations.

(3) By crosses between the lines it was shown that the number of first generation progeny depended chiefly upon the female (presumably upon the number of eggs laid) and that the second generation progeny were more numerous than any of the parent line averages, owing to the heterozygotic condition of factors controlling offspring number.

(4) Tests made at 29.5 C. showed that only the original wild stock and two of the crossed lines could breed at that temperature for more than two generations, and that only the two weakest of the inbred lines could not survive more than one generation.

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A COMPARATIVE STUDY OF THE CHROMOSOMES OF MAMMALS[1]

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INTRODUCTION

DURING a series of studies upon mammalian spermatogenesis the writer has made careful counts of the chromosome number in at least one species in seven out of the nine eutherian orders. The primary object of these investigations was to discover whether or not sex is determined in man and other mammals, as in the invertebrates, by definite sex chromosomes. An accurate knowledge of both the form and the number of the diploid chromosomes was, of course, a very important link in the chain of evidence needed to prove the presence of such sex determiners in any animal. Once it was firmly established, however, that sex chromosomes occur in two such extremes as the marsupials on the one hand and the primates on the other, this question of sex determination no longer entirely dominates our interest, and other phases of mammalian cytology appear in the foreground as progress is made in the study of the chromosomes of this group.

At the present time mammalian chromosome studies, aside from the question of sex determination, have both a practical and a theoretical interest and value to biologists. From the practical side the exact determination of both the form (especially size relations) and the number of chromosomes of domestical animals gives the cytological foundation upon which the geneticists and practical breeders of the future must build. Of the many theoretical aspects, perhaps the one of dominant interest centers about the behavior which the chromosomes have shown during the evolution of the several mammalian orders. Is there any evidence to show that very marked and extensive changes in the chromosomes have occurred which might have played a rôle in the evolution of these smaller groups? This general question is receiving a great deal of attention from students of plants, and many botanists of to-day are seeking to explain the formation of new species among plants by relatively gross changes in the chromosome complex. Classic examples of this are *Oenothera gigas,* which is a tetraploid race of *Oe. lamarckiana* and *Oe. lata,* which has one more chromosome than *Oe. lamarckiana* and is thought to have been formed from the latter by the addition of one chromosome which presumably arose in meiosis through the non-disjunction of one of these elements. Equally as striking are the observations of Blakeslee and Belling in the Daturas. Numerous other cases are cited and discussed in two recent papers by Gates (24a, 24b) and even at this early time there seems to be no question but that polyploidy and the duplication of one or more chromosomes have played rôles, perhaps very important ones, in the formation of new species among plants.

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[1] Contribution No. 195 from the Department of Zoology, University of Texas. The writer has been aided in this work by grants from the Committee for Research on Sex Problems, of the National Research Council.

The eutherian mammals are such a well-unified group that few zoologists doubt that they have arisen from a common stem. This means, of course, that this common stem must have carried the same complement of chromosomes and of genes within the chromosomes. As this ancestral stock diverged into the nine orders we know to-day, how have the primitive chromosomes behaved? Will we find that certain or all of the chromomere associations have been very stable, so that we will be able to recognize the same chromosome in different orders? Or, on the other hand, have some of the chromosomes been lost, or broken up, or fused, or perhaps undergone both of the latter processes with the formation of entirely new associations of chromomeres and of genes? Have either polyploidy or the duplication of one or more chromosome pairs played a rôle here? At the outset it would be impossible to predict an answer to any of these questions or whether or not extended researches would give us even a hint with regard to them.

There will doubtless be some who will regard this evolutionary aspect of the chromosomes as extremely problematical since, especially in the insects, there is so little apparent order in the great maze of cytological data which has accumulated over a period of thirty years. But the insects, in addition to being extremely old phylogenetically, have undergone a degree of specialization which we do not find in the mammals. Many of the insect orders were fully established long before the mammals appeared on the earth, and while in our classification we regard the mammals, in a general way; as roughly equivalent to the insects, they are really more nearly comparable to an insect order. And within these smaller groups of insects, there is a basic similarity of the chromosomes, as the works of McClung and his students have shown in case of the Orthoptera and Metz and others in the case of Diptera. The extreme youth of the mammals, as compared to some of the insect orders, would lead us to expect that the mammals would prove favorable for determining the general trend in the behavior of the chromosomes during the evolution of this class, if going hand in hand with differentiation into the different orders there has been any uniformity in chromosome behavior.

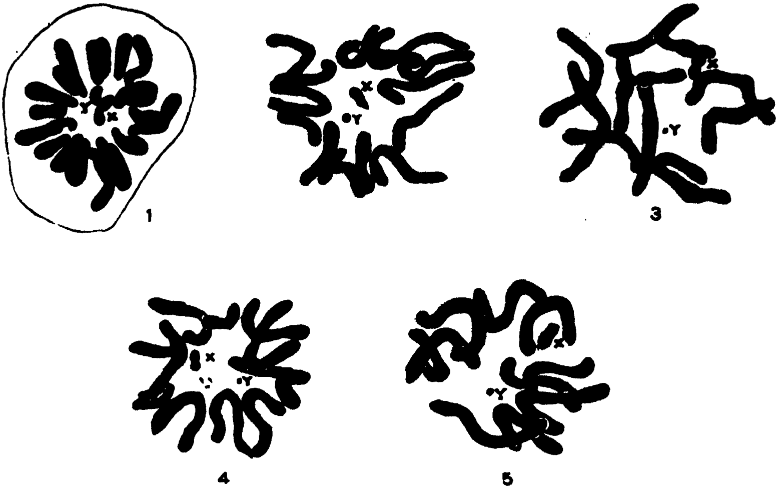
During the past year the writer has been able to make accurate chromosome counts in three different eutherian orders (insectivors, rodents and edentates) and we now know the chromosome constitution of at least one species in all the orders except the Sirenia and Cetacea. It has seemed wise to bring together in one place and to discuss from the standpoint outlined above the results which have been obtained during the past five years. I fully realize that many of the questions before us can not be answered with finality, but a discussion of the facts will enable us to see the present status of mammalian chromosome work, and will indicate, perhaps, the lines of study which it will be most profitable to follow in order to gain more light on the general problem of chromosome behavior during evolution.

MARSUPIALS

The marsupials have proved to be the most favorable mammals for chromosome studies, and published works include detailed descriptions of six species from four different families. The number of chromosomes, as compared to the eutheria, is invariably a low one, ranging from 22 in the opossum (Didelphys) to 12 in the kangaroo (Macropus). In Figs. 1 to 5 the spermatogonial chromosomes of five different species are shown. It is to be noted especially that the magnification at which the figures are reproduced is not the same in all cases. I have purposely enlarged the drawings of Agar and of Greenwood, as noted in the legend of the figures, until the size of the X chromosome was about the same for all the species shown.

In the opossum (Fig. 1) there are 22 chromosomes, 20 of which typically form a ring surrounding the small X and Y sex chromosomes. This is the highest number so far encountered in the marsupials.[2] In Phascolarctus (Fig. 2) there are 16 elements, and here again, the large autosomes form a ring about the sex elements. Sarcophilus (Fig. 3) and Dasyurus (Fig. 4) both have 14 chromosomes. In Macropus (Fig. 5) there are 12 elements; this last being the smallest number so far recorded for any mammal.

[2] Agar (*l.c.*) found 22 chromosomes in Petauroides, but since no spermatogonial cells were given in this work, we have been unable to compare this species with the opossum.



Figs. 1 to 5. The spermatogonial chromosomes of five different marsupials are represented, as follows: 1. opossum, 2. Phascolarctus, 3. Sarcophilus, 4. Dasyurus, and 5. Macropus. Figs. 2 to 4 were copied from Greenwood's ('23) Figs. 2, 23 and 27 respectively, and Fig. 5 was copied from Agar ('23) Fig. 3. It is to be noted that these drawings do not represent the same magnification in all cases.

An examination of the figures will show that there is a marked similarity in the chromosome constitution of the marsupials. This is most evident in the case of the sex chromosomes, which are not only of the same type (X–Y) and of the same behavior during meiosis, but are similar in the bilobed condition of the X chromosome. The Y chromosome, as compared to the X element, shows marked differences in size in the different species, being largest in the case of the opossum (Fig. 1) and extremely minute in Sarcophilus, Dasyurus and Macropus. In every case the sex elements are sharply marked off from the autosomes by their small size.

The autosomes, in turn, are alike in general form, all of them being elongated elements much larger than the sex chromosomes. An exact quantitative comparison has not yet been made between either the autosomes or the sex chromosomes of the different species, but if we may use the size of the X chromosome tentatively as a rough measure of chromosome volume, it would seem

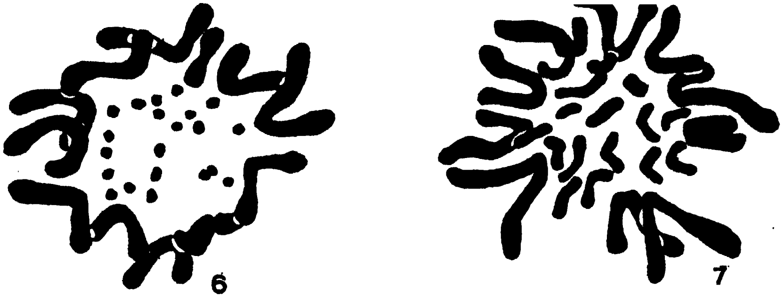


Figure 6 is a spermatogonial metaphase plate of Anolis carolinensis. Fig. 7 represents the diploid chromosome of Rana temporaria (copied from Witschi ('24) figure 3, plate XIX).

from the figures that in spite of differences in autosomal numbers in different species, the total bulk of the chromatin in marsupials is not very different. For example, let us consider the two extremes in numbers found in the opossum and in the kangaroo. Using the X chromosome as our standard of comparison, the 10 autosomes of Macropus (Fig. 5) are all much longer than the largest chromosome in the opossum, so that in spite of its low autosomal number Macropus probably carries in its 10 chromosomes a rough equivalent of the opossum autosomal complex.

Further discussion of marsupial chromosomes will be taken up later in this paper.

Eutheria

The literature dealing with eutherian chromosomes is filled with discrepancies concerning the chromosome number of most of the species studied. For example, in the case of man the reported number ranges from 16 to 48. It is now clearly recognized by cytologists that earlier workers were handicapped by an inadequate technique to handle mammalian chromosomes, and it would subserve no useful purpose to take up here the literature in detail. Since the development of an adequate technique little work on the eutherian chromosomes has been done except by the writer, so that in the following pages I shall confine

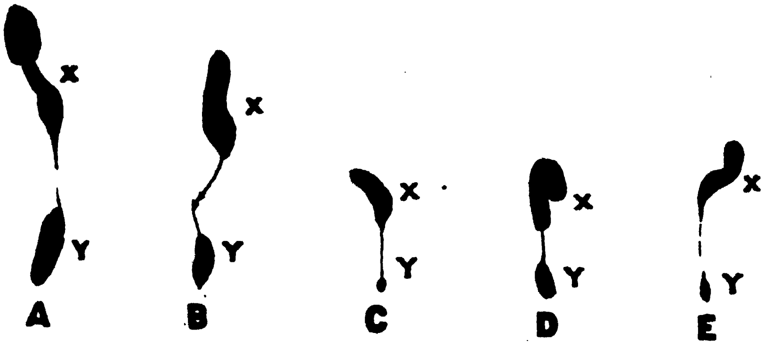


Figure 8. The sex chromosomes of various mammals are here shown as they appear in the first maturation spindle. The magnification is the same in all cases. The forms involved are as follows: A. opossum, B. negro, C. Macacus, D. Cebus, and E. horse.

my descriptions (as regards the eutheria) to those species in which I have made detailed observations. In Study I (Painter '22) a full review of the work prior to 1920 has been given.

In order to facilitate a ready comparison between the chromosomes of different mammals I have placed all the chromosomes of a cell in a horizontal line. This was done in the following way. A drawing is made of the chromosomes of a cell in the ordinary way with the aid of a camera lucida. Then the individual chromosomes of the cell are copied from the drawing on separate bits of cardboard, a copying camera lucida being used for this purpose. In this way one can place chromosomes of similar size and shape together, and beginning with the largest chromosome pair, line all the elements up in a row, approximately in the order of their size. All the chromosomes of Plate I have been drawn and reproduced at the same magnification, which is about 3,600 diameters. Two points must be stressed with regard to the figures on Plate I. In the first place, it must be realized that the pairing up of homologous elements is only approximately correct, especially with medium-sized elements, because in a given cell not all the elements will be in the same plane and one which is tipped on end will appear shorter than it actually is. I have tried to offset this source of

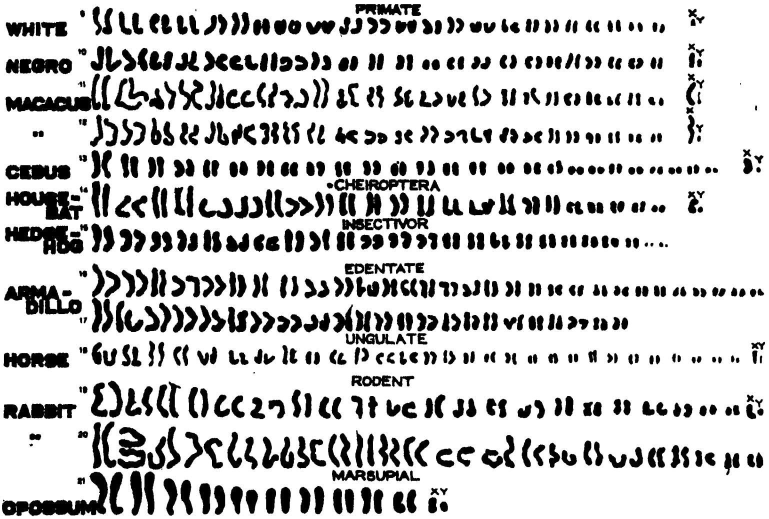


PLATE I

The magnification of all figures of this plate is the same.

error, introduced by foreshortening, by comparing chromosomes under the microscope which appear alike in my drawings. In spite of this unavoidable source of error, however, the majority of chromosomes in a given cell match up in a very striking way, as may be seen for any species represented on Fig. 1. The second point is this, that while all the chromosomes on Fig. 1 are reproduced at the same magnification, the original cells from which they were taken were often of very different sizes. Thus an amnion cell is typically much larger than a germ cell, and chromosomes taken from the former will appear much larger. On Fig. 1, the chromosomes of the bat, armadillo and rabbit were taken from dividing amnion cells, while the other figures were taken from germ cells. Again, the appearance of a given chromosome in different cells of the same individual even is affected by the rate of penetration of the preserving fluid and by slight differences in staining. If the penetration of the preservative is slightly retarded by overlying tissue, the chromosomes appear bulkier and shorter than where more rapid fixation occurs. Because of these unavoidable differences in technique and of cell size, we can not compare directly the chromosomes in different cells of the same animal or in different species. Our comparisons must be relative, as, for example, the largest pair of chromosomes, or the smallest pair, in different cells.

PRIMATES

My studies in the primates include a description of the spermatogenesis of man, both white and negro (Study II), and of a new and an old world monkey (Study IV). The diploid numbers encountered in these several forms are as follows:

Homo sapiens, white	48
Negro	48
Macacus rhesus	48
Brown Cebus	54

Homo sapiens: As far as I have been able to determine there is no difference between the chromosomes of the white man (Fig. 9) and the Negro (Fig. 10). The chromosome number is the same in both cases and a comparison of the different chromosome pairs, when these are placed in alignment approximately according to their size, shows the same gradual seriation from the largest to the smallest elements. The Y sex chromosome is the smallest body in the cell in both races, and, as I shall show below, the X chromosome is also alike in form and behavior.

Macacus rhesus: The chromosome number of this species is the same as that of man, namely, 48. The chromosomes from two spermatogonial cells are illustrated in Figs. 11 and 12. Due to exceptional preservation the chromosomes of this monkey appear more elongated than in man, but in spite of this one may see that there is the same general seriation in size in the chromosomes of these two primates. The sex chromosomes of the rhesus monkey are of the same type as found in man, the Y being again the smallest elements in the cell. The X chromosome of rhesus, as identified in Figs. 11 and 12, appears larger than the X of man. However, in maturation, as I shall show below, when the sex elements can be identified with certainty the X element in man and in the rhesus monkey is about the same size. In spermatogonia it is impossible to identify the X since it has no distinguishing characteristics, and it appears probable that either in man or in the rhesus the true X element is not represented.[3]

Brown Cebus: There are 54 chromosomes in this new world monkey (Fig. 13) including X and Y sex chromosomes. When this chromosome complex is placed in serial alignment, it is evident that as compared to man or the old world form, in cebus the small chromosomes are more numerous and there are correspondingly fewer large elements.

Sex chromosomes of primates: The figures on Plate I indicate that the sex chromosomes of the primates are of the same X–Y type. In the first maturation division, when the sex elements can be identified with certainty, it is evident that they are alike in general form and relative size and show the same behavior. In Fig. 8, B to D, these sex elements are shown as they appear in the first maturation division in man, the rhesus and the cebus monkeys, respectively.

CHEIROPTERA

The species studied was *Nyctinomous mexicanus,* the common house bat of the Austin region. The tissue used for chromosome counts was the amnion of young embryos. There are 48 chromosomes in this species (Fig. 14). When these are paired and lined up in approximately the order of their size and shape, we have a chromosome alignment very similar to that observed in man and the rhesus monkey. It will be noted that the relative size of the bat chromosomes is due to the large size of the amnion cell from which the figure was taken.

[3] As I have pointed out above, matching up of homologous chromosomes can be approximate only. It is clear that in both man and the rhesus monkey there is a medium-sized element which has no mate of like size or shape, but it is impossible to identify the true X at this time in either form.

I have been unable to identify, as yet, the sex chromosomes of the bat in maturation, but the constancy with which 48 chromosomes is found in different embryos, taken together with the fact that in some embryos a pair of very unequal sized elements is observed, would seem to indicate that we have in this form the X–Y type of sex chromosome. The embryo from which Fig. 14 was taken is presumably a male.

INSECTIVORA

The species studied in this order was the common European hedgehog. The testis was the tissue studied and has been reported upon in a short paper (Painter, '25). There are 48 chromosomes in the hedgehog, as Fig. 15 will show. When these are paired up and arranged according to size, we find the same sort of serial alignment observed in the bat and in some of the primates. In the hedgehog the two smallest pairs of chromosomes are more diminutive than in other orders with 48 chromosomes. The sex chromosomes of the hedgehog have not been identified as yet, because of a lack of proper material.

EDENTATES

The form studied in this order is the armadillo. In an earlier paper (Study II) I reported that, judging from spermatogonial counts, the number of chromosomes in this species was over 50. More recently counts have been made on dividing cells in the amnion. The number of chromosomes present in this form proves to be 60 (Figs. 16 and 17). An examination of the figures will show that there are proportionally more small chromosomes in this animal than in forms with only 48 chromosomes. I have not, as yet, identified the sex chromosomes of the armadillo, since the high number involved, together with the presence of so many small chromosomes, makes the exact study of meiosis extremely difficult.

UNGULATA

The form studied in this order was the horse (Study V) in which I found 60 chromosomes. When these elements are lined up in the approximate order of their size and shape (Fig. 18), it is evident that the small chromosomes are proportionally more numerous than in animals with 48 chromosomes. The sex chromosomes of the horse, as shown by maturation stages (Fig. 8 E), are of the X–Y type, quite similar in general form and behavior to such sex elements in marsupials and primates.

RODENTA

The amnion cells of rabbit embryos were studied in order to determine the chromosome number. Eight different embryos showed consistently 44 chromosomes. Figs. 19 and 20 give the chromosome alignment of two embryos. An inspection of these figures will show that in contrast to such forms as the horse or armadillo, or man even, the rabbit does not have many small chromosomes. The sex chromosomes have not yet been certainly identified in maturation, but embryos all carry the same number of chromosomes (44), and when these are paired up, we find that in some embryos a pair of unequal size remains (Fig. 19), while in others all elements have mates of the same size and shape (Fig. 20). The embryos with the pair of unequal sized elements (Fig. 19) have been interpreted as males with X–Y sex chromosomes.

CARNIVORA

I have studied, in a preliminary way, the testis of the dog. In spermatogonia I am able to count at least 50 chromosomes. Such counts can only be taken as close approximations, but it seems likely that the diploid number in the dog is close to 52.

DISCUSSION

The facts recorded above are of great interest because of the light which they throw on the general question of the behavior of the chromosomes during the evolution of a small group such as the mammals. If the eutheria have come from a common stem which had at one time the same chromosome constitution and genetic complex, as I have assumed in this article, then a number of questions arise concerning the fate of these primitive elements during the evolution of the various orders. These will be enumerated and discussed below. Back of these questions, however, the matter of central interest is whether or not we can find in the behavior of the chromosomes any evidence for the cause of variations. To express the matter somewhat differently we may ask, Have the changes which have occurred in mammals been due entirely to mutations within the genes, or have extensive and gross changes in the chromatic material played a rôle, such as the botanists are apparently finding in the case of many plants?

As my studies on sex determination have led me to study the chromosomes in different eutherian orders it has become increasingly clear that there is a marked similarity in the chromosome complexes of all the species so far examined. The lowest number which I have observed so far is 44 and the highest 60, but in all these cases there have been the same general size limits, and often a great similarity in the configuration of many of the individual chromosomes. In Plate I, I have brought together and arranged in horizontal rows typical chromosome complexes from species of six different orders, but before entering into a discussion of the facts brought out by this plate, let me again caution the reader that before we can compare the chromosomes from different animals we must take into account differences which are due to cell size and to technique.

The chromosome numbers which I have found in different eutheria are as follows:

Primates	*Homo sapiens* white	48
	Homo sapiens Negro	48
	Macacus rhesus	48
	Brown cebus	54
Cheiroptera	House bat	48
Insectivora	Hedgehog	48
Ungulata	Horse	60
Edentata	Armadillo	60
Rodentia	Rabbit	44
Carnivora	Dog	50 +

The average of these numbers is a fraction over fifty, but the number which I have most frequently found is 48. This is present in the most primitive eutherian order of insectivora, and in such divergent orders as the primates and cheiroptera. The occurrence of 48 chromosomes in three different orders has led me to believe and to tentatively assume that this represents approximately the primitive eutherian number.

When we compare now the chromosome complexes of different species which possess 48 elements, either within an order such as the primates (Figs. 9 to 12) or in different orders (Figs. 9 to 12, 14 and 15) it is apparent that the general seriation of the chromosomes is very similar. Beginning with the largest pair of chromosomes, we have in all cases the same gradual tapering off in sizes. The fact is that in spermatogonial metaphase plates the chromosomes of man, the rhesus monkey and the bat are so similar that one would have difficulty in distinguishing between these species. The same would be true of the European hedgehog, were it not for the diminutive size of the four smallest chromosomes.

The question now arises, Are the 24 pairs of chromosomes found in the primates, cheiroptera and insectivora basically homologous. That is to say, are the different pairs of chromosomes of the same relative size alike in that they carry essentially the same genetic elements? A final answer to this question can only be given by the geneticists of the future. When we are able to plot the chromosome-maps of the eutheria as those of the diptera are plotted to-day, then we will have the evidence needed to show that the largest chromosome pair, for example, carries the same genes (in the main at least) in different orders.

In the absence of any genetic evidence on this subject as yet, all the cytologist may do is to point out the inherent probability that these 24 chromosome pairs are alike. This does not mean that very extensive changes have not taken place within the genes themselves, but rather that the skeleton framework of the chromosomes, which is the material substratum for the genes, has undergone little morphological change in the species concerned. This conclusion rests upon two general considerations. In the first place, if 48 is about the primitive eutherian number as I have inferred (both because it occurs in three widely different orders and is near the mean of the counts made for species in seven orders) then those animals which show this number have probably retained the primitive complex. It is possible of course that individual chromosomes have undergone changes due to translocation of parts, or even by the loss of entire chromosomes which has subsequently been masked by the fragmentation of other elements, but there is no evidence to indicate that either of these processes has taken place. The chromosomes of different forms with 48 elements are as near alike as we should expect them to be when we consider the limitations in our method of comparison. The second line of evidence is that afforded by the sex chromosomes. In all the cases in which I have been able to identify the sex elements in meiosis they have been alike in type, general form and behavior. From a purely cytological standpoint the close morphological similarity of the sex chromosomes, especially the X element in different mammals, would indicate that this element has undergone little visible change. From a theoretical point of view we might expect this to be the case because, were the X chromosome to undergo a great loss of material, it would probably upset the balance between the autosomes and the sex chromatin and would result in sexually abnormal forms which would be incapable of handing on their peculiar chromosome condition to later generations.

How are we to explain the high chromosome numbers found in the ungulates or the edentates? Two possible explanations are open to us. Either there has been a fragmentation of some of the larger chromosomes, or there has been a duplication of certain chromosome pairs through non-disjunction. The first of these seems the more probable because when we compare the chromosomes of the horse, armadillo or the cebus monkey with those of man, for example, it is apparent that when there is a larger number than 48, there is an increase in the relative number of small chromosomes, and a corresponding reduction in the number of larger elements. This evidence would seem to amply justify the conclusion that the high chromosome number of the horse or armadillo has been produced by a fragmentation of larger elements which has resulted both in the increase in the total number and in the number of smaller elements. The same explanation would apply of course to the Cebus monkey.

It is impossible, with the rough standard of comparison available at present, to eliminate entirely the possibility that one or more chromosome pairs may have been duplicated in those animals with more than 48 elements. That is to say, that we may have present in these cells four homologous chromosomes (of certain chromosomes) rather than the normal two. It is well known that occasionally in maturation a chromosome pair may fail to separate (non-disjunction) so that the resulting gamete may carry more than its normal complement of chromatin. Were we to assume that this process had taken place a number of times in the horse, the high number of this animal could be accounted for. However, since fragmentation is indicated by cytological observations and is adequate to account for the facts, we need not consider the remote possibility that duplication of chromosomes is responsible for the condition encountered.

The occurrence in some forms of a smaller number of chromosomes than 48 is also open to two interpretations; either there has been an end to end fusion of certain elements or else whole chromosomes have been lost. A comparison of the rabbit's chromosomes with those of man will show that in the rabbit we do not have as many small elements as in animals with 48 chromosomes. The condition of the rabbit may be satisfactorily explained by assuming an end to end fusion of some of the autosome pairs, thereby reducing the total number to 44. This seems more plausible than that two chromosome pairs were entirely lost, though the latter should be borne in mind as a possibility and eventually tested out genetically.

Both the processes of end to end fusion and of fragmentation of chromosomes have been reported for closely related species of animals and plants. Fragmentation has figured most prominently, perhaps, in plants where it is especially common in the Liliaceae. Hance ('18) has worked out with thoroughness the case of *Oenothera scintillans*, where a fragmentation of chromosomes may occur in different individuals of the same species. End to end fusion, on the other hand, is especially well seen in the Diptera, as the work of Metz ('14) has shown. In the eutherian mammals the evidence indicates that both fragmentation and end to end fusion of chromosomes have played rôles in producing derivatives from the primitive complex.

As a general conclusion from the facts and considerations presented above, it is apparent that the chromosome constitution of the eutheria is closely similar. 48 seems to be the typical and probably the basal number for this group, and derivatives from this have been due either to fragmentation or to end to end fusion. There is no evidence which indicates that there has been any great change in the total amount of chromatin, either by additions or by losses. There is, furthermore, no evidence that polyploidy has played a rôle in species formation.

It is clearly recognized by geneticists and by cytologists that neither a fragmentation of the chromosomes nor an end to end fusion of different elements would exert any influence on the evolution of a group. Fragmentation would tend to decrease, somewhat, linkage, and bring about thereby a greater random assortment of genes, for when a chromosome breaks in two, genes located on either side of the break would be separated and the linkage formerly present would be destroyed. End to end fusion, on the other hand, would have the opposite effect, of course; at the point where chromosomes fused genes would become closely linked, whereas before they enjoyed a random assortment. The individual genes, however, would not be changed by these associations.

The marsupials have a general uniformity in their chromosome complex which is strikingly different from that found in the eutheria. Yet according to our phylogenetic conceptions the ancestors of the marsupials and the eutheria must have come from a common stem and must have possessed at one time the same chromosome constitution. This brings us to a consideration of two questions; first, what was the primitive chromosome constitution of this common ancestor, and second, what relation do the chromosomes of the eutheria bear to those of the marsupials.

The evidence which we have of the chromosome constitution of the vertebrates below the marsupial level is too fragmentary to allow us to form a reliable judgment. We know nothing about the chromosomes of the monotremes, and in the reptiles only the Lacertilia have been carefully studied.

In the lizards it is clear both from my own work (Painter '21) and that of Dalcq ('21) that we have the bulk of the chromatin localized in a small number of larger chromosomes, while a small portion of it may be scattered in a great number of dot-like micro-chromosomes. In the Iguanidae in which I have studied six species, the number of these large chromosomes is invariably 12. Fig. 6 is a spermatogonial plate of *Anolis carolinensis.* In the family Teiidae the number of the large chromosomes was higher, probably about 26. Dalcq found 19 large elements in *Anguis fragilis.*

The present-day amphibia have been extensively studied and the chromosome numbers reported have usually been 24 or 26. In *Rana temporaria,* which has recently been carefully studied by Witschi ('24), there is a tendency for the greater part of the chromatin to appear in a few large chromosomes (Fig. 7). This figure was copied from Fig. 3, Plate XIX of Witschi's paper.

If we were to generalize from the limited observations on the reptiles and amphibia, we would have to conclude that in the lower vertebrates the chromatin tends to occur in a small number of large chromosomes. However, the Anura and the Lacertilia are such specialized offshoots from the amphibia and reptilia, respectively, that the value of evidence from either is very problematical. The presence of the dot-like micro-chromosomes in the lizards suggests that changes in their chromosome constitution are under way. For the present, then, this question of the primitive chromosome condition of vertebrates below the marsupial level must be kept open. Extremely valuable evidence on this point should be given by the monotremes and by Sphenedon. It is hoped that cytologists who can obtain this material will not be slow in determining the chromosome constitution of these forms, for with the evidence of the monotremes and of the primitive reptiles before us, we should be able to postulate, with reasonable assurance, the chromosome condition of the most primitive mammals.

Turning now to the second question of the relation between the chromosomes of the present-day eutheria and marsupials, the great differences which we observe in these two sub-classes may be explained in one of two ways. It has been suggested a number of different times that the high number encountered in some of the eutheria was caused by a doubling of the chromosome number (tetraploidy) at some point in their ancestry. This suggestion has been founded, in some cases, on erroneous observations. For a long time the available evidence seemed to indicate that 24 was probably the typical eutherian chromosome number, and when higher numbers were reported, for example, 36 or 48, they were regarded as triploid and tetraploid races, no account being taken of the difficulties which would arise due to the presence of sex chromosomes in mammals. Thus when d'Winiwarter ('12) reported 47 chromosomes for a white man, and 24 had been previously reported for the negro, it was suggested that these differences could be accounted for by a doubling of the autosome number in the white man. My work clearly demonstrated later that the chromosomes of the white and negro races are alike. Polyploidy, however, is still regarded as a possible source for differences in chromosome numbers in mammals by Gates ('23, page 19). The second possible way of explaining the observed differences between the marsupials and eutheria is that the total amount of chromatin is approximately alike in these two sub-classes, but that in one case it appears in large packets, so to speak, while in the other we have smaller aggregations. The observed facts accord better with this last possibility.

In the foregoing pages I have pointed out that from a theoretical point of view we should expect the elements which determined sex to be the most stable of the chromosomes, during the course of evolution, for the reason that any great change in the makeup of the X chromosome would doubtless result in sexually abnormal individuals. That this theoretical expectation is borne out by cytological observations is apparent from Fig. 8. In this figure we have the sex chromosomes of the opossum and of four eutherian species reproduced at approximately the same magnification. It will be noted that in all cases the type of sex chromosome is the same, and considering the difference in cell size, the size of the X chromosome is not very different in forms as far removed from one another as the opossum and man. It would seem therefore that the X chromosome would afford us a rough measure of chromosome volume which will be of value in estimating the quantity of chromatin in the autosomes of the opossum and the eutheria.

In Fig. 21 on Plate I we have the spermatogonial chromosomes of the opossum reproduced at the same magnification as the other forms. Using the X chromosome as a measure of chromosome size or volume, it is clear that most if not all of the chromosomes of the opossum are very much larger than any of the autosomes of the eutheria. If we could cut up the opossum chromosomes in some way, we would probably have enough chromatin to make the eutherian autosomes. We have no way of knowing at present which is the more primitive condition, the high number found in eutheria or the low number characteristic of the opossum, but the figures make it abundantly clear that the total amount of chromatin found in animals at and above the marsupial level is not greatly different. Either fragmentation or end to end fusion, as the case may be, will adequately account for the observed chromosome constitution in marsupials and eutheria.

In the insects we do not always have a uniformity in size of the X chromosomes in related forms; in the Heteroptera the X may be very large as in Protenor, or small as in Largus (see Wilson '25, p. 755). It is obvious that we could not use the X element in the Heteroptera as a measure of chromosome size. This, however, does not alter the fact that in mammals the X is more or less constant in size and may be used as a rough standard of measure for the autosome. Just how the X chromosome in insects may vary greatly in dimensions without markedly affecting the somatic characters of the different species is not clear. It is possible that in some forms the X chromosome may carry in addition to the chromatin concerned with sex determination and sex-linked characters a considerable amount of inert chromatin (inert in the sense that it carries no genes) just as is the case with the Y chromosome in Drosophila. In the latter case the genetic evidence demonstrates that the Y chromosome is empty (of genes) although in its staining reaction it appears to behave like the other chromatin in the cell. If in some insects the X were freighted down with inert chromatin the loss of some of this would have no effect on either the sex or the somatic characters of the progeny.

I have pointed out in a recent paper (Painter '25) that the assumption that polyploidy has occurred in mammals is beset with difficulties because of the presence of sex chromosomes. There is no question but that the cytological basis for polyploidy exists in the testis of mammals and other vertebrates. Tetraploid germ cells and giant spermatozoa have been reported quite generally for the vertebrates, and I have myself recorded their presence in lizards, in the opossum and in man. In Study II (p. 309) I pointed out some of the consequences which we would expect if a giant spermatozoon were to function in fertilization. We have no reason to believe that tetraploid germ cells are confined to the male sex. It has been generally assumed that giant spermatozoa, along with other atypical spermatozoa, were not functional, but there is no apparent reason why a giant egg cell could not develop after normal fertilization. However, in the end result, it would make little difference which sex contributed the gamete carrying double the normal number of chromosomes. With the doubling of the autosomes we would have a doubling of the sex chromosomes. If the doubling were to take place in the female, then the egg after maturation would carry $2n+2X$ chromosomes, and upon fertilization with normal sperm we would have zygotes with the following constitution, $3n+3X$, or $3n+2X+Y$ (depending on which type of sperm, the X or the Y carrying individual, functioned). The $3n+3X$ individuals would be triploid females, and the $3n+2X+Y$ individuals would probably be intersexes, if we may judge from the work of Bridges ('22, '25).

If the doubling took place in the male germ cell, we would have on fertilization with a normal egg triploid individuals with the following possible sex chromosome constitutions: $3n+3X$, $3n+2X+Y$, and $3n+X+2Y$.

The further reproduction of triploid individuals with normal stock would result in a variety of different possible zygotic combinations of sex chromosomes. Bridges ('25) in a paper which has just appeared has enumerated the expected classes and indicated the sexual character of those which have been observed in Drosophila. As he has shown, tetraploid individuals may arise (females) but, even were such individuals viable, it would require rigid selection and matings to maintain a tetraploid race.

There is one additional reason why tetraploid individuals could not be maintained in mammals. During the past three years I have followed the behavior of the tetraploid germ cells of man in maturation. The point of significant interest is that during the growth period of primary spermocytes, no chromosome-nucleoli appear in tetraploid cells. Normally the chromosome-nucleolus is made up of the sex chromosome elements (Painter '24a) which retain a condensed form during this phase of spermatogenesis. The absence of chromosome-nucleoli is interpreted to mean that when two X chromosomes are present in a cell, they undergo synapsis, and behave otherwise as the autosomes do. The same thing happens apparently to the Y chromosome. We should expect that in the maturation divisions the sex chromosomes (X and Y) pairs would act independently and would be distributed as in the egg. If this were the case, each sperm would carry both an X and a Y chromosome. On fertilization with a normal egg, only one type of zygote would appear $2n + X + Y + n + X = 3n + 2X + Y$.

We may conclude, therefore, that extensive polyploidy could not have played a rôle in animals at and above the marsupial level.

In the present paper a good deal of attention has been given to chromosome numbers, yet at the same time it has been fully realized that numbers *per se* are of secondary importance. The significant point is that as far as we can gauge it, the total amount of chromatin in the different mammalian groups is about the same, and there has been a remarkable stability in the chromosome associations. Inferentially, we may surmise that the total number of genes is about the same in all groups. In their chromosome constitution the mammals have shown themselves, so far at least, comparable to an order of insects.

If my general conclusion is a valid one, then we may expect that the plotting of chromosome maps in the eutheria will go forward with comparative rapidity, because linkage values established in one group or species can be applied to other forms. For example, if genes a, b and c are located in one chromosome in the rabbit, and genes c, d and e are found in the rat, we should expect, from chromosome studies, that when genes a and b are eventually found in the rat, they will have the same position as in the rabbit. Transverse fragmentation or end to end fusion will occasionally upset these relations, but on the whole they should prove the same in different forms, and enable us eventually to plot the chromosome maps of the eutheria, although not all the characters will have been observed in one species.

Up to the present time my work has led me to make as broad a survey of the eutheria as possible. The next logical step is the intensive study of the chromosomes of different species within an order, to determine the limits to which fragmentation or end to end fusion has been carried within a small group. For this purpose I have chosen the rodents, both because of the large number of different species readily available for study, and because these animals have been used so extensively and will allow us to test out genetically the validity of the conclusions to which our cytological studies have brought us.

At the present time a number of the common rodents are being studied at this laboratory, but a host of other species not available to us in Texas can be readily obtained and studied at other universities in this country. With the technique I have used in my studies, the preservation of mammalian tissue presents no great difficulty and making chromosome counts becomes a matter of care and patience. It is hoped that other cytologists will become interested in the rodents so that we may have an extensive knowledge of the chromosomes within this group.

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THE REDUCTION DIVISION IN RELATION TO MUTATION IN PLANTS AND ANIMALS[1]

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THE reduction division has been the subject of much study on the part of geneticists and morphologists, for it constitutes one of those critical phases which are of fundamental importance in connection with biological theories, particularly those having to do with heredity. The meiotic or reduction mitoses are much easier to study in plants than in animals and are moreover much more constant in time of appearance. As a consequence of this situation, an enormous amount of information has accumulated on the botanical side regarding peculiarities of the maturation or reduction divisions particularly in relation to hybridism. It will be shown in the present article that the reduction division in plants and animals which have been used in recent years, as examples of the origin of species by mutation, is often abnormal and in essential features duplicates the peculiarities found in the meiotic mitoses of known hybrids.

To Rosenberg belongs the credit of having first clearly called attention to the meiotic peculiarities of hybrids in plants. His study of the cytology of reduction in a hybrid between the long-leafed and the round-leafed sundew, *Drosera longifolia* and *Drosera rotundifolia,* is now a classic. This sterile hybrid is not uncommon in northern Europe and passes variously under the names *D. ovata* or *D. anglica.* In this hybrid there is a very interesting situation present in the reduction division. As Rosenberg pointed out, the metaphase of mitosis in the heterotypic division is characterized by the presence of ten double (bivalent) and ten single (univalent) chromosomes. According to Rosenberg, the ten double chromosomes represent the ten gametophytic chromosomes of *D. rotundifoia* associated with ten of the twenty gametophytic chromosomes of *Drosera longifolia.* The ten univalent chromosomes represent the surplus chromosomes from the *D. longifolia.* Further striking peculiarities of mitosis in this classic example are the lagging of the chromosomes in their movement towards the equatorial plane (metaphase) and a further lagging in their progress towards the poles (anaphase). This lagging is characteristic of the smaller or univalent chromosomes. Another interesting feature found in the Drosera hybrid is the fact that from few to several of the univalent chromosomes are so tardy in their movement that they are frequently left out of the daughter nuclei. The result of this situation is the formation of small subsidiary nuclei. The final consequence may be the formation of more than the normal four pollen grains from each mother cell. The additional grains are smaller in size and owe their nuclear equipment to the lagging chromosomes.

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[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

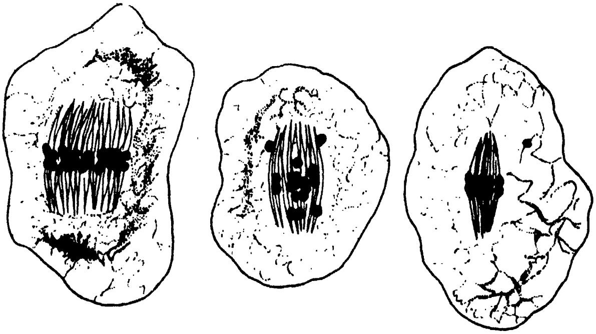

FIG. 1

Since the appearance of Rosenberg's epochal contribution to the cytology of hybrids, many publications have recorded similar phenomena in other known hybrids. In a summary article such as the present, it will be unnecessary to indicate these in detail, particularly as an admirable résumé of the literature has recently appeared in

Tischler's "Allegemeine Pflanzenkaryologie," Berlin, Verlag von Gebrueder Borntraeger, 1922.

It will serve a useful purpose at the present stage to indicate, in a general way, studies which have been carried on in recent years on the cytology of extremely variable large genera of plants. Rosenberg's work on the genus Hieracium is of fundamental value in this connection. He has shown that the species of Hieracium are characterized by different equipments of chromosomes and that these chromosomes behave in the fashion described above in the case of *Drosera ovata,* a hybrid between *D. rotundifolia* and *D. longifolia.* A very extensive and thorough investigation in this direction is furnished by Taeckholm's work on the European species of Rosa.[2] It has been shown here that the genus Rosa, although it has normally seven chromosomes, may have multiples of these up to eight fold; in other words, there are diploid, triploid, tetraploïd, pentaploid, hexaploid and octoploid roses. The roses with the multiple equipments of chromosomes usually present not only a high degree of variability and sterility but also the characteristic meiotic phenomena of hybrids, such as supernumerary nuclei abortive pollen grains, lagging chromosomes both in metaphase and anaphase, bivalent and univalent chromosomes, etc., etc. Blackburn and Harrison have likewise carried on investigations on the roses with essentially the same result as those of Taeckholm and Rosenberg. In my own laboratory, Dr. A. E. Longley has investigated the cytology of numerous species of Rubus (blackberries) and Crataegus (hawthorn), with conclusion that many of these represent natural hybrids on the basis of their meiotic phenomena which closely resemble those found in the hybrid *Drosera ovata.*

It will be convenient in this connection to call attention to the meiotic phenomena in a cryptogamic hybrid. Not uncommonly in clear lakes one finds gigantic and sterile examples of the genus Isoetes, the quillwort. These gigantic forms have many more leaves than the normal species and are much more vigorous vegetatively, often exceeding in dimensions three or four times those found in the parent forms. Fig. 2A represents the late metaphase of the heterotypic division in a hybrid species of Isoetes found in the Lakes of Law, Cape Breton Island, Canada. Fig. 2B shows the early anaphase in the heterotypic division. Fig. 2C shows the homotypic mitoses. In the item on the right of C is shown the early anaphase, whilst on the left is represented a later stage of the anaphase. In all the cells shown in Fig. 2 are represented conditions such as are characteristic of known hybrids. These are respectively the occurrence of two categories of chromosomes, large and small, and the marked lagging of the chromosomes, particularly those which are smaller in size, both in the metaphase and the anaphase, and in both the heterotypic and the homotypic mitoses. These gigantic forms of Isoetes as represented by the particular form under discussion are extremely sterile and the microspores are frequently entirely abortive, whilst the megaspores vary greatly in size and present striking ab-

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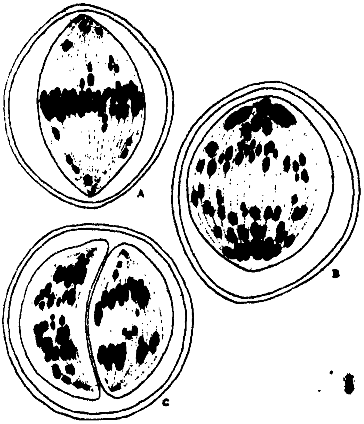
[2] *Zytologische Studien ueber die Gattung Rosa,* von Gunnar Taeckholm, Uppsala, 1922.



normalities. This hybrid, which will be fully described elsewhere, is apparently a cross between *Isoetes echinospora* and *Isoetes lacustris,* as it is found associated with these two species. The gigantic individuals always resemble the lacustris parent, since they possess its characteristic habit, stiff texture and reticulately sculptured megaspores.

With these preliminary statements in regard to known hybrids and polymorphic species which resemble in all their essential cytological features hybrid forms, we may pass to the consideration of certain so-called mutating types. The genus Oenothera has been put forward as an example of the origin of species by mutation by the Dutch physiologist, de Vries. He observed that in cultures of *O. lamarckiana,* a very small fraction of the seedlings differed from the parent form. The various aberrant types originating in this way were sometimes very sterile and always more or less sterile as is also the case with the parental *O. lamarckiana.* It was found that these abnormal derivatives bred true to type and they were considered by de Vries as a consequence to represent elementary species or mutants. It has been generally conceived by those who adopt the doctrine of mutation that these elementary species are the result of a spontaneous change in the germ plasm of *O. lamarckiana.* It was found subsequently that other species of Oenothera had the same peculiarities as *O. lamarckiana* and in some of the American species the production of aberrant mutants is at times very large, amounting in some instances to 90 per cent. of the seedlings. Bartlett has proposed for this marked variability the phrase "mass mutation." In an interesting series of investigations, Davis[3] has shown that it is possible to produce artificially and by hybridization an Oenothera closely resembling *O. lamarckiana* to which he has given the name *O. neo-lamarckiana.* This interesting artificial hybrid resembles *O. lamarckiana* in breeding relatively true, generation after generation, and also in throwing in cultures a relatively small number of elementary "species" or "mutants." There has been during the past two decades an enormous amount of activity in the investigation of the cytology and genetics of species of Oenothera. It is very generally agreed at the present time by geneticists of prominence that *O. lamarckiana* and other so-called mutating species of Oenothera are of hybrid origin. This conclusion is largely confirmed by the cytological conditions found in the heterotypic meiosis.

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[3] "The Behavior of Oenothera neo-lamarckiana in selfed line through seven generations," B. M. Davis, Proceedings of the American Philosophical Society, Vol. 63, No. 3, 1924.

It will now be convenient to take an example from the cryptogams illustrating the process of mutation. Many years ago in a greenhouse in Cambridge, Massachusetts, there originated by chance a fern which is now known as the Boston fern. This is a derivative of the West Indian species *Nephrolepis exaltata* as shown by the fact that it originated in the same greenhouse with it. The Boston fern is very generally regarded by mutationists as a mutant of *N. exaltata.* This fern is distinguished by two features: first, by its almost complete sterility and second by its great vegetative vigor and adaptability to unfavorable conditions. The second feature has made it very popular as a decorative and a house plant. This fern shows a high degree clonal variation and the clones have originated from time to time a huge number of varieties, which have been exhaustively studied by Benedict,[4] who has put forward the view that the Boston fern exemplifies the origin of species by mutation. In one of the clones of the Boston fern known as variety *fertilis,* a certain number of spores are capable of germination and it has been recently shown by Benedict that these spores give rise to a very varied offspring in contrast to the parent species *N. exaltata* in which the sporelings are very uniform.

The great vegetative vigor and hardiness of the Boston fern as compared with its tropical progenitor, its ex-

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[4] *Journal of Heredity,* 1924, and list of literature.

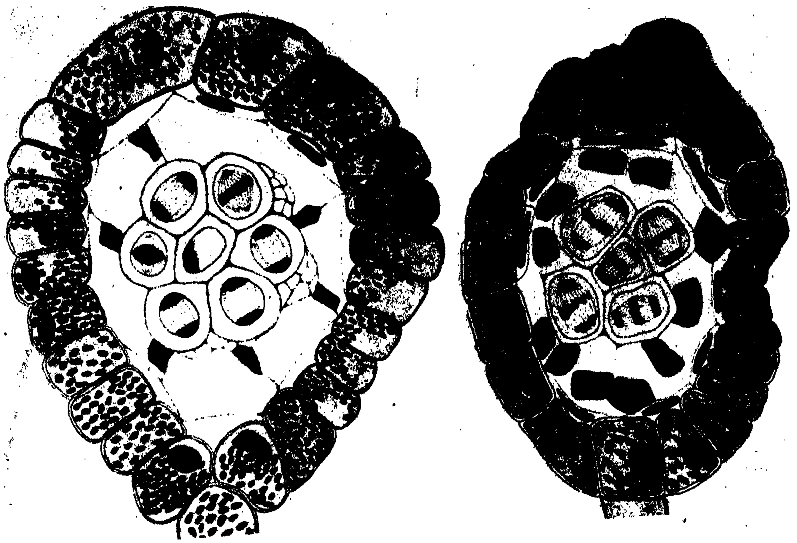

FIG. 3

treme variability, both in clones and sporelings, and finally its marked sterility are all indicative of a hybrid origin. The detailed cytological investigation of this polymorphic species will be described elsewhere, but it will be of interest in the present connection to introduce illustrations of the heterotypic mitosis in the parent *N. exaltata* and *N. exaltata* variety *bostoniensis*. Fig. 3A represents a sporangium of *N. exaltata* in which the spore mother cells are undergoing the first or heterotypic division. The illustration is on such a scale that it will be possible for the reader to recognize that the maturation divisions, both in the metaphase and anaphase, are entirely normal. *N. exaltata,* even when grown in greenhouses, produces a large number of spores which are apparently entirely normal in structure. Figure 3B illustrates sporangium of the Boston fern in the same stage of development, from a plant grown in the same greenhouse and on the same bench as the *N. exaltata* figured above. It will be obvious on inspection that the reduction or meiotic division in this sporangium is quite abnormal and is characterized by the presence of lagging chromosomes such as make their appearance in known hybrids. The sporangium, moreover, appears somewhat shrivelled in contrast to the rotund outline shown in the previous figure. This shrinkage is a precursor of later abortive development, for normally in the fully developed sporangia there are no spores capable of germination, since all are shrivelled and usually without any protoplasmic contents.

It is thus obvious that the Boston fern has not only all the vegetative characteristics of a hybrid, such as extreme vigor and reproductive sterility, as well as great variability (in all these features it presents a marked contrast to the parental *N. exaltata*); but also the detailed investigation of the crucial meiotic phenomena in this fern indicates very clearly a hybrid (heterozygous) origin. It will thus appear, to all but those extremists, whose minds are blind to all but experimental evidence, that the Boston fern is a hybrid derivative of the tropical *N. exaltata*. It is not difficult to imagine such a mode of origin because hybrid ferns originate very frequently in greenhouses where a number of species of ferns are cultivated together.

It will now serve a useful purpose to call attention to the meiotic phenomena of insects, a group of animals very closely connected in their evolution with that of the higher plants. Fig. 4 shows the metaphase of the meiotic mitoses of a number of insects as taken from the recent third edition of Wilson's classic, "The Cell in Development and Heredity." A, B and C represent the spermatocytic divisions of three genera of beetles, namely, Trirhabda, Chrysochus and Photinus. D, E, F and G show the same condition in four Hemiptera, namely, Protenor, Leptoglossus, Anasa and Pachylis. Obviously in all these heterotypic metaphases designated above, the chromosomes are arranged in pairs end to end (telosynapsis) and exactly in the equatorial plane. In other words, the illustrations picked at random from Professor Wilson's admirable work show that the normal conditions in the

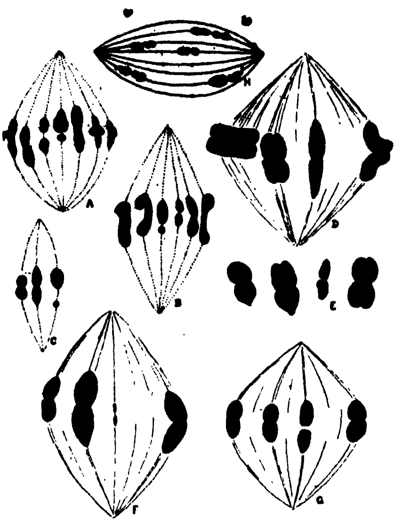

FIG. 4

critical or maturation mitoses in insects exactly resemble the corresponding conditions in the flowering plants.

With this preliminary statement, we may now pass to the consideration of the Pomace fly, *Drosophila melanogaster*. This dipterous insect has been the zoological counterpart of *Oenothera lamarckiana,* and a whole school of biology has practically been founded on the study of this extremely variable type. The discovery of its variability is apparently to be credited to Professor Castle,[5] of Harvard University. Later, Professor Morgan, of Columbia University, exploited the species in connection with the theory of mutation on the zoological side and a tremendous amount of activity has developed, particularly in genetical laboratories, in the direction of a study of the chromosomes and genetical characters of the Pomace fly. In all contemporary genetical textbooks it occupies a prominent position and is considered to exemplify and prove the origin of species by mutation. This situation is all the more remarkable because Oenothera has now passed for the most part into the biological discard, and very few, even among the geneticists, consider its peculiarities to be due to anything else but a hybrid origin.

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[5] *Journal of Experimental Zoology,* 1906.

A noteworthy feature of the cytological study of Drosophila, a genus to which the greatest importance is attached by mutationists, is our almost complete ignorance in the past of the all-important phases of the meiotic mitoses. It is true that Miss Stevens has given some attention to this subject, but, as was pointed out later by Metz, her methods were crude and unreliable. As a consequence, we have, up to the present time, no accurate information regarding meiotic or reduction divisions in *Drosophila melanogaster.* This is a surprsing condition in view of the great theoretical burden which has been put upon the chromosome apparatus in Drosophila. Morgan, in his recent summary of "The Genetics of Drosophila" (Bibliographia Genetica Deel II, 'S-Gravenhage, 1925) notes the difficulties connected with the study and interpretation of the meiotic or reduction divisions in this species. He states that they are both hard to investigate and to interpret.

Investigations carried on by the present authors seem to indicate that the employment of rapidly penetrating preservatives simplifies greatly the technical difficulties connected with the study of meiosis in Drosophila. The use of Carnoy's absolute alcohol, chloroform and acetic acid, the penetration of which is facilitated by a powerful air pump, brings about a very satisfactory general condition of preservation. This reagent is particularly valuable on account of its solvent action on the fat which is present in such large quantities in the body cavity of the pupae and which often constitutes an envelope surrounding the reproductive organs and shutting them off from the rapid action of preservatives. The fat-dissolving chloroform in Carnoy's solution successfully overcomes this obstacle to penetration.

The cytological investigation of the pupae, which are most satisfactory for the study of the meiotic phenomena in the testes, has made clear that the reduction division in *Drosophila melanogaster* presents the identical pecu-

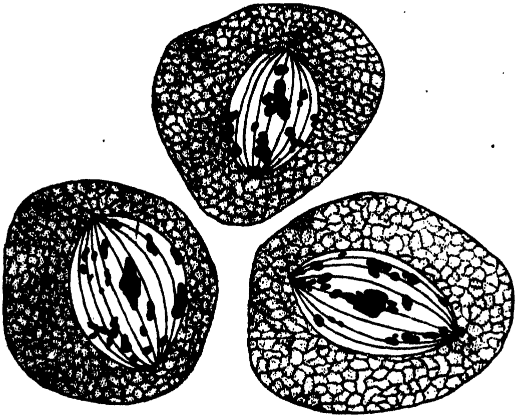

FIG. 5

liarities which have been noted above as characteristics of hybrids. The failure of Morgan and his associates to interpret properly the phenomena of meiosis in *Drosophila melanogaster* is in all probability due to their unfamiliarity with the literature dealing with the cytology of hybrids, particularly that of hybrid plants which is very large and important. Fig. 5 shows the formation of the spindle in the meiotic mitosis of Drosophila as exemplified by a large number of preparations which have passed under our observation. It is obvious that the chromosomes are irregularly distributed, after the manner of hybrids. Certain of the larger ones occupy a position near the equator of the spindle, whilst numerous smaller ones are distributed towards the poles. We have here a condition which corresponds to the early metaphase in divisions in normal species of insects. The irregular distribution of the chromosomes and their very large number are noteworthy features. By mass methods of sectioning, we have secured quantities of the relatively rare mitoses in the testes of Drosophila (wild, scarlet, spineless, brown and white). Later, the chromosomes tend to group in twos, and a notable number of them, especially in the later stages of mitosis, are cast out of the protoplasm. A more advanced metaphase is

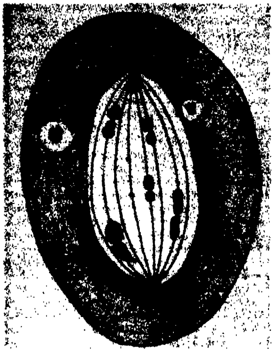

FIG. 6

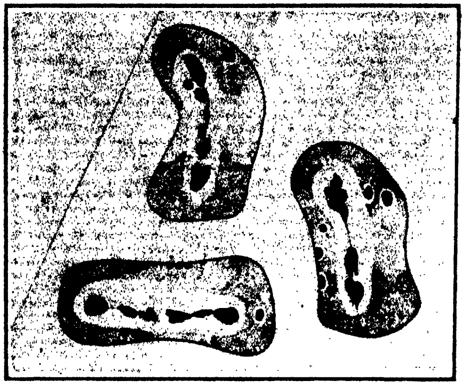

FIG. 7

shown in Fig. 6. Here the chromosomes are clearly paired end to end and lie along the threads of the spindle. We have obviously here to do with the synaptic stage of the metaphase. An interesting fact, however, is the failure of the synaptic mates to arrive simultaneously in the equatorial plane. We have never observed in the investigation of many thousands of preparations a single equatorial plate in which the synaptic mates are grouped in the normal fashion for insects. The conditions in fact, on the contrary, are distinctly those found in hybrids and certainly even if this interpretation is not accepted, the conduct of the chromosomes in meiosis is highly abnormal. Not only are the chromosomes abnormal in their conduct in Drosophila, but in the metaphase, particularly in its earlier conditions, are far more numerous than would correspond to a theoretical statement of Professor Morgan and his associates. They assert that there are four chromosomes, haploid, and eight chromosomes, diploid, in *Drosophila melanogaster*.

The anaphase of mitosis in the meiotic division of Drosophila is equally interesting. In Fig. 7 are shown a number of spermatocytes which are in this stage. The reader will observe that there are approximately eight chromosomes in the region of the spindle and that these tend to form two polar groups of four each. The illustra-

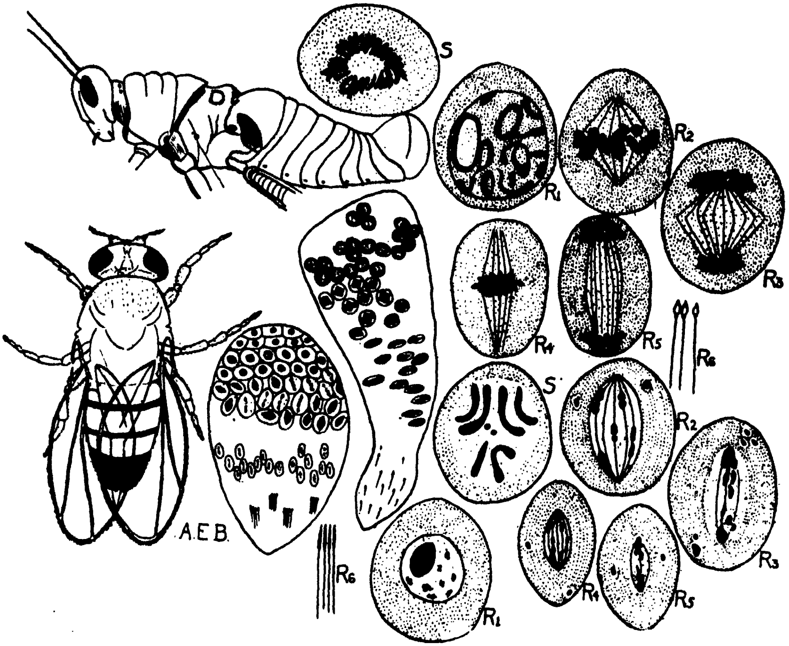

FIG. 8

tion shows a more regular condition than that which frequently occurs. Very generally the larger chromosomes reach the poles in groups of approximately four, leaving behind them large numbers of chromosomes of much smaller size in the intervening region. Another feature of interest in the anaphase is the passage of a larger or smaller number of chromosomes into the protoplasm also shown in Fig. 7.

It will be obvious to the reader who has followed the statements and illustrations appearing in previous pages that both the metaphase and anaphase of the meiotic mitosis (reduction or maturation division) in *Drosophila melanogaster* are distinctly of the type found in hybrids. Even if extreme mutationsists should refuse to grant this resemblance to be of significance, it is quite obvious that the highly important meiotic phenomena in Drosophila are entirely abnormal and aberrant. These abnormalities may be catalogued as follows: Polyploidy or unexpectedly numerous chromosomes, lagging of the chromosomes, absence of an equatorial plate and finally the elimination of many of the chromosomes in the later anaphase into the protoplasm of the cell.

If one adopts the comparative attitude in regard to these phenomena, one is obliged to consider at the very least the strong probability of the hybrid origin of *Drosophila melanogaster.* The mitoses in its meiotic divisions resemble, for example, quite strikingly those in such a well-known hybrid as the mule, and have their abundant counterpart in the very numerous data presented by the cytological phenomena of hybridism on the plant side. Of course, if one takes the point of view that the comparative and structural study of plants and animals is out of date, these facts would have no significance except as abnormalities. It appears pertinent, in this connection, to bring into court the close relationship between chemistry and physics. The physicist, for example, who attempted to draw any serious conclusions from the spectroscopic study of material, which had not been passed upon previously by the chemist, would be open to the serious charge of inaccuracy and superficiality. Similarly, it appears obvious that the physiologist or the geneticist who disregards the fundamental structural characteristics of the material with which he works is likewise open to severe criticism. Not a few of our physiologists and geneticists at the present time seem to merit the description aptly bestowed by Mr. Kipling on the personages of the psychological novel, "They steam for steaming's sake . . . their port is all to make."

If one takes the attitude that only experimenal data are of any importance in biological investigations, the point of view apparently adopted by many extreme experimentalists, the result will inevitably be extreme sterility in the biological field. Living beings are distinguished, above all, from inanimate matter by the capacity for reproduction and inheritance. These distinctive phenomena are largely connected with structures and obviously structures can not be left out of sight in any theory of inheritance no matter how well founded experimentally. It is clear, however, that mutationists of the Morgan school have not rejected structures in the study of heredity, since they attach the greatest importance to the chromosomes and their significance as the bearers of hereditary characteristics.

If we follow the geneticists of the Morgan school in evaluating chromosomes as of high importance, and if we go further than the members of this school have yet gone in bringing into court also the crucial and critical meiotic phenomena which they have practically entirely neglected, we are compelled to conclude that the Pomace fly is thoroughly abnormal in its maturation or reduction divisions and that the abnormalities present a detailed and striking resemblance to those presented by known hybrids. The only reasonable concluson from this situation is that *Drosophila melanogaster* is a hybrid and that both its extreme variability and its remarkable meiotic divisions are the result of this fact. It follows, of course, that the huge and top-heavy superstructure which has been erected by the extreme school of geneticists on the too fragile basis provided by the Pomace fly falls of its own weight. This is not surprising, as similar course of events has already presented itself in the pendant case of so-called mutation on the plant side, namely, *Oenothera lamarckiana.*

It will serve a useful purpose to draw a clear parallel between the divisions of the cells in the spermatogenesis of such a type as the grasshopper, which is commonly used in the laboratory for illustrating the general principles exemplified by the mitotic divisions in the sperm-mother cells of animals, and those found in the Pomace fly, which has been used as the basis of a theory of evolution by so-called mutation. Fig. 8 in its upper part shows a diagrammatic illustration of a male grasshopper. Above the insect is represented one of the somatic cells with its large equipment of chromosomes. To the right, R. 1, it shows the spermatocyte getting ready for its first division and exhibiting a notable number of the ring chromosomes found in the Orthoptera, etc. R. 2 shows the metaphase of the first division of the spermatocyte. R. 3 illustrates the anaphase of the same division. It will be noted that the chromosomes are quickly and sharply transferred to the equator and to the poles, respectively, in the metaphase and anaphase. R. 4 and R. 5 show the corresponding phases of the second division of the spermatocytes, and R. 6 illustrates on a smaller scale of magnification three spermatozoids, such as result from the final divisions of the spermatocytes. Below the figure of the grasshopper and to the left of the spermatocytic divisions described above is shown a diagrammatic view of one of the segments of the testes of the grasshopper. In the upper region appear spermatocytes with ring chromosomes. Below these are spermatocytes in the first division. Lower still are found the second divisions of the spermatocytes and in the lower region of the lobule are represented fully developed spermatozoids.

The figure in the lower left-hand corner of the illustration represents a male Pomace fly (*Drosophila melanogaster*). To the right of it is shown a diagram of one of the testes. In this are exhibited the reproductive cells in the various stages of development corresponding to those of the grasshopper. Fig. S to the right of the testis of the grasshopper contains the well-known diagram of the chromosomes in the somatic cells of a male *Drosophila melanogaster*. R. 1 shows a spermatocyte in the developmental stage. In R. 2 is represented the metaphase of the first division of the spermatocyte. In R. 3 the anaphase of the same division is depicted. In both these figures it will be noticed in contrast to the situation in the grasshopper that there is no assemblage of the chromosomes in the equatorial region and at the poles in the definite manner found normally in dividing spermatocytes. It is further noted in R. 2 and R. 3 that there is an extrusion of chromatin from the nucleus into the protoplasm. In R. 4 and R. 5 are shown the metaphase and anaphase, respectively, of the second spermatocytic division. In R. 6 appear the spermatozoids resulting from the second spermatocytic division.

The diagrammatic illustrations in Fig. 8 make it clear that the spermatocytic or meiotic divisions of *Drosophila melanogaster* are fundamentally different from the normal conditions found in the corresponding cells of the grasshopper. It has been shown above that the geneticists, who have made a special fetish of Drosophila, have failed to notice this abnormality in the meiotic phenomena. Having failed to notice the aberrant maturation, they have naturally failed to offer any interpretation of the peculiar conditions present. It is of importance to note that the meiotic mitoses of the Pomace fly present a striking and inescapable resemblance to the conditions which present themselves in the reproductive cells of plant and animal hybrids. It follows that the theoretical conclusions drawn from the exhaustive study of *Drosophila melanogaster* must be fundamentally revised. *Drosophila melanogaster* obviously presents an example of a phenomenon which is extremely common on the plant side, namely, the appearance of a natural hybrid. The evidence grows continually that a large number of what are called species in plants are not pure-blooded forms but hybrids.

THE OCCURRENCE OF AN EYE AND OF A TOOTH ABNORMALITY IN A LINE OF ALBINO RATS

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It has been demonstrated many times, in those forms whose eggs are fertilized outside the body cavity of the female, that deviations from the normal oxygen supply are capable of producing metabolic changes in the developing embryo. Since such changes result in visible disturbances of the growth of certain organs, one is led to believe that their effect might be more fundamental than the observed external anomalies, and that the germ plasm might also be influenced. This experiment was undertaken with the dual idea of attempting, by this method, to alter the developmental rate of mammalian embryos directly, and to discover if, at the same time, a more fundamental metabolic change were produced which might affect the germ cells.

The experimental procedure is given, and the behavior of the stock recorded. It should be stated at the outset that no cause and effect relationship is claimed between the experimental procedure and the results obtained. These results are given in order that other investigators may know of their occurrence. The experimental method is explained with the idea of adding another possible approach to the problem of experimental modification of the germ plasm.

The experimental production of abnormalities in those forms whose eggs are either laid or developed outside the body of the female has been a fertile field of investigation. Stockard (1921) reviewed the work which had been done up to that time from the viewpoint of developmental rate and structural expression. His theory to account for the abnormalities is based on the fact that every individual varies slightly from every other individual due to differences in development. The variations are noticed when they appear in exaggerated form, and are then recognized as anomalies. The propositions advanced are: that all types of abnormals or monsters may be caused by the same experimental treatment; that any single type may be caused by a great number of different treatments; that all effective treatments tend primarily to lower the rate of development; and that the type of monster induced depends on the particular developmental moment or moments during which the developmental rate was reduced.

In a given species there is a specific rate of development at which the early cleavage stages follow one another, and at which the formation of different organs begins. This may be due to the chemical structure of the egg and to the oxidation rate of the material in the egg. Although in the later stages the amount of food available influences the rate of development, the supply of oxygen and the temperature have a more striking effect.

The two methods most commonly employed to induce alterations in the developmental rate are: temperature changes and oxygen deficiencies. No experiments with mammals are recorded in which these methods of directly influencing prenatal development were consciously employed.

The work with mammalian material has been done chiefly with the hope of affecting the germ plasm. The experiments of Stockard (1912, 1913) with alcohol on guinea pigs; of Guyer (1918, 1920) with antibodies in rabbits; of Little and Bagg (1923, 1924) with X-rays on mice; and of Hansen (1924) with alcohol and X-rays on rats may be mentioned here. In all these cases an outside agent, chemical or physical, was employed as an "activator." In undertaking the present investigation, the plan was to attempt an alteration in the normal rate of development of the embryo by some method which would cut off the oxygen supply at a critical time. It was thought that this could be accomplished by delaying implantation. There was thus the possibility of obtaining two reactions: an external one, due to an upset in development; and an internal one, due to the absence of sufficient oxygen for normal growth, with the possible accumulation of carbon dioxide.

Stock sent us from the Wistar Institute as Line A Selection stock was employed.

METHODS

In order to obtain an adequate control, in addition to the use of the Line A Selection rats, a litter of young was first obtained under normal conditions from a given male and female. These young were bred inter se and kept as a control line for comparison with subsequent litters from the same parents after treatment. After the weaning of this control litter, the female was examined daily by the smear method described by Long and Evans (1920) for determining stages in the oestrous cycle. When the smear indicated that the female was in heat, she was again placed with the male, and the mating observed. The female was then put in a cage by herself, under the same environmental conditions, and with the same food as the other rats of the colony.

Following the schedule worked out by Long and Evans (1920) for the position of the fertilized egg at different stages in its development, the fourth day after fertilization was selected as the most favorable time at which to attempt to produce some metabolic change which would affect the blastula. At this time, the fertilized egg has presumably reached the uterus, but has not yet become implanted. The initial oxygen supply of the egg may, therefore, have become nearly exhausted, and the means of reaching its next supply not yet established, since that is to come, after implantation, from the maternal blood. If at this stage, then, implantation could be delayed, the oxygen supply might be impaired, if not absolutely cut off, and an alteration in the metabolic rate induced. Subsequent implantation might then occur, metabolic equilibrium be restored, and development continue.

OPERATIVE TECHNIQUE

Of the various methods tried at the beginning of the experiment, only one, that which was employed in the majority of cases, will be described here. The female was etherized, and a lateral, dorsal incision made. Without severing any of the connections, the ovary, Fallopian tube and part of the uterus were then lifted out of the body cavity, being held with forceps, and exposed to the air for five minutes during which they were cooled and dried by a gentle fanning. They were then replaced in the body cavity, and the incision closed with two or three stitches. In some cases, this operation was performed on the right side, in others on the left, and in a few animals on both sides. The rats recovered within a short time. They were closely watched for the next two or three weeks, and the effect of the operation noted. In some cases, pregnancy appeared to be interrupted, while in others it was apparently normal, and was terminated with normal parturition.

RESULTS

Twenty-seven females were treated experimentally. Six of these were etherized only, in order to test the effect, if any, of that part of the operative procedure. Of these, five produced normal litters, whose subsequent offspring were all normal; one produced no young. Six other females were subjected to more drastic types of operation. Only two of these produced offspring, all of which were normal and produced only normal young. Fifteen females were treated by the technique described above. Of these, ten produced offspring, and five failed to become pregnant.

Of the ten experimental litters, only one showed any abnormality in either the first (treated *in utero*) generation, or in several succeeding generations in which close inbreeding was practiced. The one female (female 69) who was the mother of the first abnormal eyed individual, had the left ovary and tube exposed and fanned for five minutes. Three young were born twenty-two days after the observed mating, two of which, a male and a female, had abnormal eyes. Female 69 was mated twice after this litter to the same male (male 16) who had sired the first litter. Thirteen young were born in each litter, and one individual in each of these litters became abnormal in one eye during the course of its lifetime. They were not noticeably abnormal at birth, and certainly not at weaning. Later experience has shown us that there are often borderline cases, where the abnormality is so slight as to be barely discernible. Since there had been no opportunity to observe the degrees of the reduction in the size of the eyes at this time, it may have been that these individuals did have a slight variation at birth, which was not detected because of inexperience.

Female 69 was also mated with her abnormal son, male 99, producing four litters, totalling nineteen young. All these were normal. These lines continued for four generations and produced no abnormals.

From the three litters (including the one *in utero* at time of treatment) of female 69 by male 16, four lines have developed. Line I is from the mating of the first two abnormals inter se—female 101 by male 99. Three litters were produced, in the second of which the first two "tooth abnormals" occurred. Two subsequent generations were obtained from this litter, but no abnormals, either eye or tooth, developed. No young were obtained from the third of these litters. The first litter, however, was mated (brother by sister) and this line was carried to the sixth generation (from the treated parent). One hundred and eighty-six young were born, of which four had abnormal eyes. These were all in the same subbranch, one appearing in the fourth generation and three in the fifth.

Line II developed from the mating of abnormal female 101 by normal male 148, a sib in a later litter from female 69 by male 16. This branch was carried to the sixth generation by brother to sister matings. One hundred and fifty-seven young were born, one of which, a male, in the fifth generation, was abnormal (eye). Three litters, totalling eleven young (all normal) were obtained from him by his sister. A female from one of these litters was backcrossed to him, producing six young, two of which developed abnormal teeth. Four litters were obtained by brother to sister matings from this cross. Of these twenty-three individuals, eight have abnormal eyes. This line is being continued at the present time.

Line III was formed by brother to sister matings within the second litter obtained after treatment from female 69. This line was carried to the fourth generation with 126 young produced, and although one of the first generation females used for breeding was abnormal, no abnormal animals appeared in the progeny.

Line IV is the result of crossing the five females in the third post-operative litter from female 69 by male 16, with their sib (in a previous litter), abnormal male 99. Four of these females were normal. The branches from them were carried to the fourth generation with 112 young, one of which had abnormal eyes.

The fifth female in Line IV was herself abnormal eyed, the condition not becoming noticeable until she was adult. Like her normal sisters, she was also mated to abnormal male 99. Two litters of young were produced, totalling fifteen individuals. All were normal. The first litter was continued by brother to sister matings, giving rise to seven litters with twenty-seven young. In one of these litters, two abnormals developed—male 949, abnormal eye; and female 951, abnormal teeth. Three of the other litters were continued by the same type of mating, but among their seventy-eight offspring no abnormalities developed.

Male 949 was mated four times to his normal sister, female 950. Among their twenty offspring, two eye abnormalities were found. These occurred in different litters. Male 949 was also mated to his abnormal (tooth) sister, female 951. Twenty-seven young were born, one of which developed abnormal teeth. The litter mates of this abnormal individual were inbred by brother to sister matings, producing eleven litters, totalling forty-three individuals, twelve of whom had abnormal eyes, and three of whom had abnormal teeth. It is worthy of note that four of these litters consisted of only two young, and in each case both of the young were abnormal in one or the other of these respects, and in one case in both respects. An effort was made to make as many abnormal by abnormal matings as possible among these individuals, regardless of whether the abnormals were litter sibs or not. Three such matings were made. One resulted in four young, two of which were abnormal; one in seven young, one of which was abnormal; and one in six young, one of which was abnormal. In one case, an abnormal-eyed female was backcrossed to abnormal male 949, producing four litters, totalling twenty young, six of which had abnormal eyes, and two of which had abnormal teeth. In two other cases normal females were backcrossed to abnormal male 949. These matings produced normal progeny. In one litter, a normal female was bred to her abnormal brother, producing four young, all of which were abnormal.

From three of these litters in which there were abnormals, the next generation has been obtained, twenty-nine young having been born. Thirteen of these are abnormal. This generation is not yet completed.

Sibs (but not litter mates) of the parents of abnormal male 949 produced five litters, totalling forty young, one of which was abnormal. This abnormal female was mated to male 949, but all their offspring were normal, and no abnormals developed in the next generation or in a backcross generation.

SUMMARY OF RESULTS

		Normal	Abnormal
Line I	F_2	24	2
	F_3	29	0
	$F_{3\,1-2}$	22	0
	F_4	73	1
	$F_{4\,1-2}$	14	0

Line I	F_5	24	2
	$F_{5\ 1-2}$	15	0
	F_6	16	0
		219	6
Line II	F_2	7	0
	F_3	60	0
	F_4	77	0
	F_5	45	1
	F_6	11	0
	$F_{6\ 1-2}$	4	2
	$F_{7\ 1-2}$	16	8
		220	11
Line III	F_2	73	1
	F_3	31	0
	F_4	21	0
		125	1
Line IV	F_2	58	1
	F_3	115	4
	F_4	124	3
	$F_{4\ 1-2}$	0	9
	F_5	43	15
	F_6	13	8
	$F_{6\ 1-2}$	27	6
	F_7	16	10
	$F_{7\ 1-2}$	10	3
		430	67

DISCUSSION

From the pedigree shown here, it is very evident that a complex situation is involved. In attempting a solution, it may be well to start with a process of elimination, and to consider first the various possibilities to which the observed conditions might be due:

(1) The abnormality might have been present in the stock before being subjected to experimental treatment. Since the rats, as stated, were Line A Selection Wistar stock, any such anomalies would have been noted in that stock at the Wistar. That there were none, Dr. King assures us. Furthermore, none were observed in the controls which were as closely inbred as the experimental lines.

(2) The abnormality may have been coincident in origin, but in no way dependent upon the treatment. The chances of such an occurrence are infinitely small, when chance alone is considered. However, this may have been the case.

(3) The remaining possibility is that the abnormality was produced by, or connected with the treatment.

The above seem to include the possible explanation of the *production* of the character.

The *nature* of the character may be considered next.

(1) It may be non-genetic. From a study of the pedigree charts this does not seem tenable. The stock of animals has been so closely inbred that the reaction of individuals from it to environmental conditions should be much the same. Furthermore, all the rats, control and experimental, were kept under exactly the same external conditions: they were fed the same kind of food, and were cared for in exactly the same manner. Were the effects, then, due to external environment, food conditions, or the like, the controls should show the same developmental responses. This has not been the case in a single instance.

(2) The character may be genetic. Considering it from a Mendelian point of view, it is clearly not a dominant trait. Neither is it a simple recessive—although in the broadest sense of the term—if the character is really Mendelian, it is a recessive under certain limited conditions.

It is not sex-linked.

In the preceding paragraphs, it is shown that we are not dealing with the ordinary types of characters. We do know, however, that some type of heredity or transmission is involved and that the method is extremely irregular. The stress up to this point in the experiment has been laid on obtaining a strain of true breeding abnormals. It was felt that until some conclusion in regard to the mode of expression of the abnormality was reached, it was useless to attempt outcrosses with unrelated strains. Therefore, no evidence can be offered at this time that cytoplasmic inheritance is not involved. In order to obtain proof on this point it would be necessary, of course, to obtain inheritance of the character through a cross with an unrelated normal female. This will be attempted as soon as it seems feasible.

On the supposition that the character is inherited, there are several interesting hypotheses which may be advanced in partial explanation of the situation. The first of these is that the presence of normal overlaps is strongly indicated, since in case after case apparently normal individuals breed as abnormals. Moreover, the recorded amount of variation in the degree of the abnormality and the delay in its development as seen in two females (♀ 144 and ♀ X) where it appeared late in life, indicate a large percentage of overlaps with the normal condition. No effort has been made to calculate such a percentage, as it does not seem probable that normal overlaps alone are responsible for all the irregularities observed.

Another hypothesis which may be considered is that the character is dependent on many modifying factors. Since the pedigrees show that the percentage of abnormals is becoming greater in the later generations, it might be claimed that as the degree of homozygosity increases, the charcter is becoming less variable. Since the original stock was highly inbred, however, and since no outcrosses with the individuals not in the experimental line have been made, this does not seem to be a sufficient answer.

The situation is apparently too complicated to be explained either on the basis of normal overlaps or of increasing homozygosity of modifying factors. If the original abnormals—affected by the treatment while *in utero*—were due to a developmental retardation from a delay in implantation, or from a disturbance of oxygen equilibrium, some explanation must be given why the same type of anomaly appears as one of the heritable or transmissible characters; if they were induced by the treatment. It is well recognized that the eye region is one of the most susceptible portions of the embryo to any environmental change, either internal or external. It is not necessary, then, to suppose that an explicit gene change has occurred or that any particular region or regions of the chromosomes, which might be responsible for the development of certain optic structures, has been affected. Under the circumstances, it seems much more probable that some metabolic or physiological condition, itself dependent on germinal constituents, has been altered, and may in turn be responsible for a delay in implantation in the embryos in each successive generation. Were such the case, either the maternal uterus, the embryo itself, or both, might be responsible for any delay which might occur. Furthermore, it is conceivable that the metabolism of either parent or embryo would vary within certain limits due to environmental fluctuations. Thus, although the germinal factors were present, they might not reach that stage of development which would cause an unfavorable physiological reaction. If, at the same time, a selective prenatal mortality of embryos were occurring, only those capable of surviving the altered internal conditions would come to birth. A second selection is also made in many cases on the basis of the breeding record. If no abnormals are produced after one or two generations from a given female, that line is discontinued. It may be that this line is potentially abnormal, but that the embryos survive the internal environment in better condition, and a larger percentage of normal overlaps is produced. Two kinds of selection are thus involved: one, due to an internal action which eliminates the grossly abnormal; and second, an artificial choosing, whenever possible, of those animals for breeding purposes of a recognizable degree of abnormality. Such an assumption would account for the larger number of abnormals occurring in the later generations.

It is interesting that the eye abnormality affects males and females alike, and apparently in equal numbers. The sex ratios of the treated and control lines are not significantly different.

Litter size shows a slight reduction in the experimental lines, falling to 5.34, while in the controls it is 6.20.

The eye and tooth abnormalities have appeared simultaneously in a sufficient number of cases to make it evident that they are not alternative expressions of the same causative agent—whether it be germinal or otherwise.

The discussion of the causes and nature of the characters here presented should not close without considering the possibility of an inherent tendency toward defects of development in this stock of rats. If there were such an innate condition toward lack of metabolic equilibrium in the stock which might have led eventually toward abnormal development without the intervention of outside factors, the experimental treatment may have had the effect of hastening the upset.

TOOTH ABNORMALITY

The tooth abnormality appeared for the first time in the second generation after the production of the eye abnormality in two individuals (a male and a female) from the same litter. It later appeared in female 951, and eight out of the ten tooth abnormals since that time have appeared among her direct descendants.

The condition is apparently the same as that described by Greenman and Duhring (1923) and by Hammett and Justice (1923). The incisors of either or both lower jaws become greatly elongated, some of them growing to form an almost perfect circle, the ends piercing through the maxilla or palatine bones. According to Greenman and Duhring, this growth is due to aberrant metabolic processes, the cases being less in number when a highly varied diet is supplied. Hammett and Justice state in their paper that the growth of the incisor teeth of normal albino rats is apparently continuous throughout life, and the reason that they do not reach a prodigious length is due largely to the fact that "an adequate apposition of upper and lower incisors ensures their grinding away their ends, as they are pushed out during growth. When such apposition is imperfect or lacking, the teeth become abnormally long. This may be due to lack of opposing teeth, deviation of the jaws or prognathism.'' In the present case, it may be that it is due to a deviation of the jaws, since the skulls of the abnormal individuals show other developmental bone defects, especially in the region of the zygomatic arch. If, however, it is due to metabolic causes, since its expression is so closely limited to one branch within the experimental lines, it is not unlikely that its development depends on metabolic conditions which are an expression of a certain germinal constitution.

That it is not a localized failure of growth control accompanying old age is shown by the fact that it has developed in several individuals at the age of six or seven months, which is the height of their early maturity.

Relation of this Work to Other Investigations

If the abnormalities recorded here are the result of the experimental treatment, then another method of attempting to influence the germ plasm has resulted in an effect on the same region that has responded to various other types of experimental procedure. In view of the different opinions held on the specificity of response to treatment, the fact that another eye abnormality has occurred is of interest.

Summary and Conclusions

(1) An eye and a tooth abnormality which occurred in the Line A Selection stock of albino rats from the Wistar Institute are recorded.

(2) These abnormalities appeared after treatment in one experimental line, and none appeared in the controls. However, no claim is made that they have been *induced* by the treatment.

(3) The method of inheritance is irregular, the occurrence of normal overlaps complicating the situation.

(4) An inherited tendency toward delay in implantation of the fertilized egg is one of the suggestions offered as a possible cause of the eye abnormality.

(5) The experimental lines are being continued by close inbreeding in the hope of obtaining lines in which there is either no occurrence of normal overlaps or a predictable percentage of overlaps.

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THE RÔLE OF BUMBLEBEES IN THE POLLINATION OF CERTAIN CULTIVATED PLANTS[1]

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I. INTRODUCTION

ALTHOUGH bumblebees make very delicious honey, they usually store it in such small quantities, as compared with hive-bees, that it would not be practicable to keep them for this purpose. However, since Darwin (1841) came to their defense, eighty-four years ago, it has been shown that these industrious insects play an important and in some cases indispensable rôle in the pollination of many cultivated plants. While this fact, as we shall see later, is sometimes grossly exaggerated, there have been, on the other hand, several attempts to minimize or completely deny the economic importance of bumblebees.

The object of this paper is to record the results of some observations and experiments which throw additional light on this question. However, before taking up the discussion of this work, it seems desirable to review briefly the interesting "history of insect pollination."

The first one who clearly recognized the importance of insects in the pollination of flowering plants was the German biologist Kölreuter (1761).[2] About thirty years later, this important discovery was confirmed by Sprengel (1793) who, in addition to greatly extending the list of insect-pollinated plants, called attention to various remarkable floral structures and mechanisms and advanced

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[1] Contributions from the Entomological Laboratory of the Bussey Institution, Harvard University. No. 236.

[2] Although it was known long before that the production of figs depends upon a small hymenopterous insect (*cf.* Hegardt, 1744; and Howard, 1899), it apparently did not occur to any of the earlier biologists that similar relations might exist between other insects and plants.

the theory—rather startling[8] for that time—that not only these, but the flowers themselves, are divine contrivances to insure insect pollination. Although some of his observations were later directly or indirectly confirmed, Sprengel's (1793) "theory of flowers" was rejected by most biologists (*cf.* Darwin, 1862; and Müller, 1873) and was in danger of being completely forgotten, when the Darwinian theory of evolution suddenly placed his observations in a new light. Inquiry into the relations between insects and flowers now became one of the most popular biological studies, and resulted in a phenomenal growth of this line of investigation, notably through the work of Darwin (1862 and 1876), Hildebrand (1865), Axell (1868), Delpino (1868–1869), Müller (1873 and 1881), Kerner (1876), Robertson (1888–1897), MacLeod (1893–1894) and Knuth (1898–1905). After a later brief period of comparative inactivity, the study of the ecology of insects and flowers has recently entered upon a new era which promises further important achievements for this branch of biology (*cf.* Knoll, 1921–1922; and Clements and Long, 1923).

II. BUMBLEBEES AND THE FERTILITY OF RED CLOVER

In Chapter III of his "Origin of Species," Darwin makes the following statement concerning the pollination of clover by bees:

> From experiments which I have tried [*cf.* Darwin (1858)], I have found that the visits of bees, if not indispensable, are at least highly beneficial to the fertilization of our clovers; but humble-bees alone visit the common red clover (*Trifolium pratense*), as other bees can not reach the nectar. Hence I have very little doubt, that if the whole genus of humble-bees became extinct or very rare in England . . . red clover would become very rare, or wholly disappear.

A few years later, this conjecture of Darwin (1859) was attacked in a series of papers by the American botanist, Thomas Meehan (1870, 1875, 1876a, 1876b), who asserted that the fertility of clover is not dependent upon bees, or other insects, to any material extent.[4] Probably as a result of this adverse criticism, Darwin (1876) subjected this question to further experimental tests, and found that a hundred flower-heads of *Trifolium pratense* which he covered with a net did not produce a single seed, while a hundred others which were visited by bees yielded several thousand. But even this did not convince Meehan (1877a, 1877b, 1878, 1884), who now contended that Darwin's (1876, p. 361) results were due to differences in nutrition and not to insects. However, in 1885—three years after Darwin's death—there occurred an event, now well known to biologists, which convinced even such skeptics as Meehan (1893).

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[8] In accordance with the anthropocentric viewpoint prevalent during the pre-Darwinian era, it was generally believed—even until quite recently (*cf.* Ogle, 1870)—that the fragrance and the various colors and forms of flowers were especially created for the purpose of pleasing the human senses.

For many years the farmers of New Zealand, especially those of the South Island, had been obliged to import all their red clover seed, because of the fact that this important forage plant would not produce seed freely in their own country,[5] a condition which was changed almost over night by the successful introduction of bumblebees.[6] In addition to this important confirmation, Darwin's (1876) final conclusion regarding the relation of bumblebees to red clover is further directly or indirectly corroborated by the observations—and in some cases experiments—of Haberlandt (1880), Beal (1887), Pieters (1896), Kirchner (1905), Fruwirth (1906), Kuhnt (1908), Lindhard (1911 and 1921), Sladen (1912), Lovell (1918), Folsom (1922), Friese (1923), Pellett (1923) and others.

However, within the last few years, Darwin's (1859) position in regard to this question has again been assailed, this time by the Austrian biologist Heikertinger (1919), who bases his criticism on the following statement of Knuth (1898):

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[4] Similar, though less sweeping, assertions were later made by Armstrong (1883) in regard to red clover in New Zealand, but this investigator seems to have placed too much faith in the rather superficial observations and experiments of Meehan (1875).

[5] This has been denied by Armstrong (1883), but his assertions are directly or indirectly contradicted by those of Dunning (1886), Thomson (1891) and Hopkins (1914).

[6] Not long ago, a similar lesson was very forcibly impressed upon some of our western fruit-growers who wasted large sums of money and much valuable time before they discovered that the Smyrna fig can not be profitably grown in this country without the insect (*Blastophaga grossorum* Gravenhorst) by which it is pollinated in the Old World (*cf.* Howard, 1899 and 1900).

> Allerdings sind die Hummeln die hauptsächlichsten (nicht, wie Darwin meinte, die einzigen) Befruchter des roten Klees, doch bleiben nach Ausschluss derselben noch immer zahlreiche, normal saugende und Pollen sammelnde Insekten, um die zur vollen Fruchtbarkeit nötigen Fremdbestäubungen zu besorgen; es ist mithin das Glied der obigen Kette: "Je mehr Hummeln, desto fruchtbarer der rote Klee" unhaltbar.

In reply to this criticism, it may be stated, first of all, that both Knuth (1898) and Heikertinger (1919) have overlooked the fact that Darwin (1876) later subjected this question to further investigation, the results of which were published more than twenty years before the appearance of Knuth's (1898) criticism. In this report, Darwin (1876, p. 361) very clearly states his final conclusion concerning this matter in the following words: "It is at least certain that humble-bees are the chief fertilizers of the common red clover."

But even if we disregard this oversight on the part of Knuth (1898) and Heikertinger (1919), we are still confronted with the important question, whether or not it is true, as Knuth (p. 295) contends, that there are plenty of insects, besides bumblebees, which insure the complete fertility of red clover. While it is possible that Knuth's (1898) assertion may apply to a few restricted areas (*cf.* Knuth, 1897; and Franklin, 1912–1913, p. 203), there is, as we have seen, a large body of evidence which indicates that Darwin's (1859 and 1876) maxim, "the more bumblebees, the more fertile red clover," is probably true of most parts of the world.

In this connection, the following observations which the writer made in the vicinity of Boston from June, 1921, to November, 1924, seem worth recording. Throughout the summer and fall of these four years, large numbers of bumblebees could always be found working on red clover, unless the weather was very inclement. In addition to the bumblebees—of which fourteen species occur within the city limits of Boston—a female of some of the solitary bees[7] and a few butterflies were occasionally found engaged in this work; but most of these visit the flowers in such a desultory fashion, as compared with bumblebees (*cf.* Clements and Long, p. 68), that the red clover in the vicinity of Boston would probably set little or no seed if its pollination depended upon other insects than bumblebees.

In regard to the hive-bee, concerning whose relation to red clover there has been considerable controversy (*cf.* Pellett, 1923), it may be stated that during the whole course of these observations not a single individual of *Apis mellifica* was seen on *Trifolium pratense,* although large numbers of them were working on alsike (*Trifolium hybridum*) and white clover (*Trifolium repens*) which was growing among or near the red clover. However, the writer does not wish to deny by this statement that *Apis mellifica* may occasionally obtain nectar and pollen from *Trifolium pratense.*

III. Experiments with the Hardy Garden Larkspur

Like red clover, the hardy garden larkspur (*Delphinium cultorum* Voss) is classed as a typical bumblebee flower, for it is claimed that the members of the genus *Delphinium* are largely, or wholly (*e.g. D. elatum*), dependent upon bumblebees for pollination (*cf.* Müller, 1873; Knuth, 1898, and Lovell, 1918). In the case of *Delphinium consolida,* Darwin (1876) found that plants covered with "a thin net" produced only about half as many seeds as those pollinated by bees.

In order to determine the effect of the exclusion of bumblebees upon the fertility of *Delphinium cultorum* Voss, the following experiments were carried out by the writer. On the 19th of August, 1923, fifty-four terminal racemes of *D. cultorum,* each having about a dozen large buds, were enclosed in paper bags so that bumblebees would be excluded from the flowers. A few days later, Professor Edward M. East, of Harvard University, called the writer's attention to the fact that in some cases this method of enclosing flowers keeps plants from setting seed properly. On August 25, five large plants of *D. cultorum,* each having a considerable number of racemes in various stages of development, were therefore completely enclosed in 1/16 inch mesh wire cages, after the number of flowers and buds of each raceme had been recorded on cards. All the enclosed racemes—including those in the paper bags—were harvested on September 29. The results of these two experiments are shown in the following table:

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[7] *E.g. Andrena wilkella* Kirby, *Halictus forbesi* Robertson, and *Megachile melanophea* Smith; det. Dr. J. Bequaert.

TABLE I

Experiment No.	Number of racemes having buds only	Number of seeds expected[8]	Number of seeds produced
1	54	42,768	17
2	35	27,720	21
Totals	89	70,488	38

The importance of insect pollination in the case of *Delphinium cultorum* Voss is also very strikingly illustrated by the three accompanying figures.

It will be noticed that these results differ considerably from those which Darwin (1876) obtained in his experiments with *Delphinium consolida,* but approach very closely to what one should expect in the case of *Delphinium elatum,* which is regarded by Müller (1873) and Knuth (1898) as being incapable of self-pollination. The few seeds which were obtained in the case of *D. cultorum* might very well be due to accidental pollination by small ants, of which large numbers were observed on the flowers of some of the enclosed plants. A similar explanation may account for Darwin's (1876) results, although it is, of course, possible that *Delphinium consolida,* unlike *D. cultorum* and *D. elatum,* is capable of self-pollination.

In the vicinity of Boston the flowers of *Delphinium cultorum* Voss are pollinated largely, if not exclusively, by

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[8] Estimated at the rate of 22 seeds per follicle and 36 follicles per raceme.

The above figures represent parts of three racemes which were used in experiment No. 2. At the time they were enclosed (August 25, 1923), the flowers of these three racemes had reached the following stages of development: Fig. 1. All the flowers had been in bloom for several days; Fig. 2. The flowers began to bloom on August 26; Fig. 3. Only the lower flowers were in bloom.

bumblebees, of which *Bremus* (*Bombus*) *affinis* and *Bremus fervidus* are the most common visitors. The last-named species obtains the nectar of *D. cultorum* in the normal way, but the workers, as well as the queens, of *Bremus affinis* invariably secure it by perforating the spur, and hence probably play no part in the pollination of the plant.

Later in the summer, bumblebees, other than the males and workers of *Bremus fervidus*, are rarely seen on *D. cultorum*. We have here, therefore, at least during a certain part of the year, a similar state of affairs as in the case of *Delphinium elatum* which, in certain parts of Europe, is said to be entirely dependent upon *Bremus hortorum* for pollination (*cf.* Müller, 1873; and Knuth, 1898).[9]

IV. BUMBLEBEES AND THE TOMATO CROP

Not long ago the following account appeared in one of the Boston papers under the heading, "Bumblebees wanted, or the tomato crop will fail":

> Bumblebees are wanted to save the tomato crop of the Northwest truck growers. There is a shortage of these black, fuzzy fellows this summer, which according to botanists are necessary in the work of pollination of the tomato blossom.
>
> Truck growers and gardeners report to the State Department of Agriculture that the fruit of the tomato will not set because of some sort of blight affecting the blossom, which falls soon after opening.
>
> The theory advanced by the ranchers is not correct. The blossom falls because the bumblebee, a specialist in the system of correctly scattering pollen, has for some unknown reason disappeared this season.
>
> It is reported from several localities in the Northwest that field mice prey on bumblebees and devour the eggs while in the comb.

The assertions in the preceding quotation are contradicted by the results of Fink (1896), who found that the flowers of the tomato (*Lycopersicum esculentum* Miller) pollinate themselves if "confined under a screen." This is corroborated by the results of some experiments[10] conducted for another purpose during the summers of 1923 and 1924 by Professor C. T. Brues, of Harvard University, who kindly permitted the present writer to make use of these data. In the course of Professor Brues's experiments, various Solanaceous plants, including two varieties of tomato, were enclosed in 1/16 inch mesh wire cages, and yet these tomatoes, as well as several plants of the genus *Solanum*, produced a large crop of fruit.[11]

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[9] In the eastern Pyrenees, Bonnier (1879) found this plant also visited by other bees.

[10] These experiments are being conducted with the aid of a grant from the Elizabeth Thompson Fund.

V. SUMMARY AND CONCLUSIONS

(1) Darwin's maxim, "the more bumblebees, the more fruitful red clover," probably applies to most, if not all, parts of the world.

(2) The hardy garden larkspur (*Delphinium cultorum* Voss) is dependent upon insects for pollination and—in the vicinity of Boston—is pollinated largely, if not exclusively, by bumblebees.

(3) The tomato crop does not depend upon pollination by bumblebees, but it is probable that the yield is slightly increased if the plants are cross-pollinated (*cf.* Bouquet, 1915).

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[11] Since writing the above, Professor Brues has called my attention to some experiments by Bouquet (1915), who found that the yield of the tomato is slightly increased by cross-pollination.

[12] In order to keep the bibliography within reasonable bounds, it was thought best to omit some of the works cited in the text. They may be found by consulting the extensive bibliography in Knuth's (1898–1905) "Blütenbiologie."

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TRIASO-JURASSIC PLANT EVOLUTION AND CLIMATE[1]

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THE geologic lapse of time considered with respect to plant change and climate lies between the stressed close of the Permian and the equable environments of the Lower Cretaceous forests. Measured in years, this period is about the equivalent of Cretaceous-Tertiary time, that is, from eighty to one hundred million years or longer. It is the time of change from a merely ancient rather than archaic plant world into that of to-day. For post-Jurassic time is only an era in which modern types fix themselves in view, and then by much further evolution amongst visible types enter a great course of dispersal and also displacement of all the more conspicuous coniferous, ginkgoid and especially cycadeoid vegetation which it is here wished to scan for clues to climate.

Taking all time from the earliest plants down, in some arbitrary sense, the age of algae is followed by the archaic plants of pre-Devonian and Devonian time; the ancient canopies of the Carboniferous and Permian end in the transition types leading into present-day vegetation during Triaso-Jurassic time; and finally the rise and dispersal of the purely modern types take place in Cretaceous-Tertiary time. That is, these terms or ideas stand juxtaposed to the great geologic and climatic eras. The era of primordial algal plants is the Algophytic, extending up into Cambrian time; of the early land plants leading into the well-marked forests of the Devonian, the Eophytic; of the upper Devonian types the Archeophytic; of the better known floras of the Mississippian, Pennsylvanian and Permian, the Paleophytic; of Triassic-Jurassic time the Mesophytic; of Cretaceous and later time the Cenophytic.

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[1] Presented at a symposium on "Ancient climates" held in connection with the meeting of Section E, American Association for the Advancement of Science, Washington, D. C., January 2, 1925.

Naturally and fortunately there are two apposite viewpoints from which these larger eras may be examined for the facts bearing on fossil climates; although even so, study must for long years to come largely end in the dialectics of inference. First, there is dominance as represented in the successive collections of fossil plants or the primary facts of geographic distribution; and, secondly, there is the much more recondite field of progressive evolution to scan—the actual advance in leaf, stem and floral types. But whichever way the investigator may turn he soon faces unscalable heights as yet lost in the clouds of doubt, and even of fancy. And yet the data of age and area, using these terms in the simple dictionary sense, are being extended; while the greater facts of specialization and extinction are being slowly disentangled from the history of the leaf, the stem and the flower as measured in the types that have prevailed. Especially, too, no one now thinks of any other than a continuous process of change in which for every more visible crest of the wave there must be the beginning and the subsidence, with lateral limits leading toward yet other crests. And were the view continued far enough some of those crests would be found small and local, others of great area. Although that which remains hidden in whatever partakes of the nature of pulsations or waves in plant change as in a sense correspondent to the diastrophic view of continental and climatic evolution, reaches immensity.

In confining attention, then, to some given stretch of climatic time it seems as much needed to search for the evidence of persistence of type as for the high lights of change. Paleobotanic science may be fairly claimed to have progressed at least that far. That is, in seeking for the data of fossil climates every angle of approach must be considered, and all that is inferential must be eliminated or at least set in its proper place. Unless the general extent and nature of change are both held in view fossil climate can not be well adjudged. And then, when the evidence from stems, leaves and flowers is somewhat brought into view, perhaps we may better judge whether Triassic-Jurassic climate was as unchanging and profoundly tropical as long thought, or whether this was just a period of ordinary earth-climate.

DICOT STEM STRUCTURES

Fortunately for the normal development of scientific ideas, through which the final divination and proof of scientific truth may be attained, even smaller groups of workers soon reach complementary hypotheses. And these quickly bring into view the more irreconcilable data. Thus all present theories of dicot stem structure rest on very limited evidence—are beset by two utterly opposed views of conifer stems, and likewise two views of magnolia stems, which are worldwide apart. There is no certainty that Araucarians are the most or the least specialized of cone-bearing gymnosperms, no accord as to whether Trochodendron, Drimys and the magnolias are primitive or not. An acceptable theory of dicotyl stem structure is plainly of the future. When agreement is reached it will mean that much more evidence has been assembled, that the manner in which dicotyl stems were evolved is virtually seen.

Nevertheless, I shall not hesitate to say I view the Araucarians to be old as pines, that I believe the resemblance of Trochodendron wood to a hard pine is real, that the resemblance of the pitted wood of Drimys to that of cycadeoids and araucaroids is also real, and not due to any such veiled course of secondary change as a general later fusion of pits into scalariform markings. And I believe, too, that most of the failure amongst botanists to agree in their interpretation of structure origins here is due to persistence of many old lines and a far more intense parallelism than a slender fossil record can possibly reveal.

Accordingly, it is suggested that from the close of the Permian to the opening of Cretaceous time, change in stem structure from the basal gymnosperm complex into the dicotyls occurred in somewhat the following order: (a) shortening of tracheids, (b) frequent segmentation of scalariform into circular pitting, (c) pith reduction to a certain extent with development of medullar and radial storage tissue, (d) merely relative enlargement of short types of circularly pitted tracheids with loss of borders, and actual reduction of adjacent elements to the fiber tracheid, (e) a course of wood ray development, (f) development of extreme branching, the sparse-leafed shoot and later leaf and floral change.

Differentiation of vessels especially has had some attention from Bailey, and appears related to a very general course of tracheidal shortening long under way. Incidentally, the great tracheids of the seed fern Lyginopteris with their multiseriately bordered pits have some of the features of vessels. The pits are indeed radial, but tangent pitting is not unknown in older woods.

The cordaite-conifer-dicotyl view of descent of a few years since left very great structure gaps to fill. But as discovery has gone on this view has given way in the minds of many botanists to other views, such as the gnetalean view, and especially the cycad or the conifero-cycadeoid view with the corollary of ancient lineage. And of course these later views mean consideration of more varied types, so that the dicotyl stem, just as in the case of the flower, is less a mystery than earlier. The gap to bridge is not so great, and discovery of the stem structure of the Triassic *Wielandiella* and of the Jurassic *Williamsoniella* is eagerly awaited. These stems must approximate very nearly a type of stem, with at times certain conifer features as well, which was very widely distributed at the break of the Trias, and which was then actually changing toward true dicotyl structure. Towards the close of the Jurassic the greater course was about ended in defined dicot stems. Thus, in the lower

Cretaceous *Aptiana* the small vessels scarcely disturb the regular radial sequence, are little larger than wood fibers and nearly as elongate as normal tracheids. The medullary rays—those leading out from the pith, have squarish cells like cycads, while scalariform wood is a striking feature in not only *Aptiana* but several of the group of Cretaceous forms described by Stopes.

Closing this brief word, it may be confidently asserted that the generalities to which any discussion of dicotyl stem origin must be confined will soon be replaced by evidence. It is only recently that the older lignites have been successfully sectioned, and it is amongst the many Triassic and Jurassic lignites that the abundant dicotyl record yet lies hidden. The conifer stems have seemed so ubiquitous as to suggest a sole and only record. But this is clearly due to their resinous character, which for centuries prevents decay and seems to aid in silicification. And then too so many of the petrified conifer forests are the great and specialized pure stands which from Triassic times down lived on low plains on the edge of the desert where easily engulfed by drifting sands with abundant silica. One of the world's greatest petrified forests for all time, that of the Chinle above the Shinarump in Arizona, has both extent and persistence in time. There the pure stands of araucarians reaching 200 feet high are petrified for hundreds of miles around as if in a full totality, and the entire surroundings, with every geologic feature as noted by Gregory indicate the edge of the desert. And those southwest deserts of a warm temperate climate, as so well marked at the beginning of the upper Trias are again noted at the break of the Jura. But with their great xerophyllous pure stands facing the desert as the Argentine araucarians face the desert and the scrub to-day in behind the Patagonia plateau, they seem to have continued almost unbroken from Shinarump to Como time, leaving behind petrified forest after petrified forest of conifers. And it was during that long stretch of time and desert, in all likelihood with its European counterpart, that the heavy-stemmed cycadeoids were segregated from their numerous kind and reached the stem bulk and feature stamping them as desert plants.

MONOCOT STEMS

The monocots, like the dicots, must have reached a strict modernity of stem and flower by the advent of Cretaceous time; although the direct evidence so far merely indicates that in the upper Cretaceous the monocots were diverse of family and cosmopolitan. While the negative evidence all goes to show that as Newell-Arber seems to have been the first to suggest, dicot foliage is young, the exact contrary seems true of the monocots. The fan type of leaf is of course a later instance of extreme specialization, but the simpler acuminate types are probably as old as monocot history. It is in fact not the leaves or flowers of the monocots but the stems which present to paleobotanists perhaps their very greatest enigma. Until recently no one has even ventured a suggestion of the manner in which the monocot stem may have arisen, unless as a totally discrete structure for all time. But now Noé and Wieland assume that the immersed primary wood of Heterangium suggests a pseudo-monocotyledonous structure, and that the so-called Myeloxylon may include types which have discarded their secondary wood entirely. If vessels could arise in secondary wood, why not in a stem the structural unit of which is the sheathed bundle?

Herbaceous types have been more than once hypothesized as derivatives of woody types; and it would seem little more strange if in the depths of the Paleophyte, where nature seems literally to have experimented with nearly every possible combination of primary and metaxylem, the dicots and monocots were actually in contact. The conifers, too, because of the presence of foliar centripetal wood in the lower Cretaceous Prepinus of Hollick and Jeffrey may once have had perimedullary strands like those of the older Cordaites. It is in brief an amazing fact that although the main lines of seed plants appear discrete from the oldest Paleozoic on, there has always been an essential unity of structure, a visible parallelism in the forward movement toward the later structural types. At least the monocot stem structure is called to mind in one very important chapter of the history of early stem organization in the seed plants, and from far back in time must stretch across the Trias and Jura, whether the record shows the fact or not. Were the forms more generally tropic than later? Surmise is the better answer.

GROWTH RINGS

The oft-repeated inference or even emphasized statement that absence of growth rings in fossil stems at once indicates higher average cosmic temperature ought to be weeded out of geologic texts and ought no longer be copied by botanists. That growth rings must often yield approximate data for local climatic conditions is evident. But several investigators, amongst whom should be mentioned Antevs, call attention to the fact that the present knowledge of fossil growth rings is very far from quantitative. Rings as yet afford little more than a clue to the more general character of climates back of the Cretaceous. For, although narrow or discontinuous rings occur from the Devonian down, it is only at the close of the Trias that zonation is better marked, and only towards the close of the Jurassic that the modern stem and foliage complexity is reached, with widespread and sharp seasonal response in growth rings. If environment means anything here, these definite end results might rather be thought of as inevitable because seasons have been sharp and climate zonal as long as there have been forests.[2] In the simpler types of wood before the days of marked tracheidal and especially wood ray tracheid differentiation, growth rings were slow to appear; and even now they result from special and local climates to which they are not always strongly affixed. Tropic species may lack the rings, and yet grow side by side with other types which have well-developed rings. Also rings have been seen in a South African monocot.

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[2] Scott has recently described two stems of the Calomopitys type from the lower Carboniferous of southern Scotland as *Bilignea solida* and *Endoxylon zonatum* of interest here. Both have regular zones of autumn wood, as does the related Mesopitys of the Siberian Permian. New evidence for former desert, cool or cold climates arises continually as stem types increase in number. Also, with more searching examination of older carbonized leaf imprints by "chemical methods," types with cutinized hairs, a protection against dry and cold, are found again and again.

In the upper Jurassic coniferous stems of King Karl's land (78° N. L.) east of Spitzbergen, Gothan found rings as well defined as in later Tertiary types. Gothan in this connection remarks that these rings appear in a well-represented group of Abietineans, that no associated araucarians were found, and that in the upper Jurassic of Spitzbergen as pointed out by Nathorst many Abietineans are again present—but there, with nearly total absence of cycadeous plants. There is some evidence for a later southward invasion of Abietineans.

Ring relations in the dicotyls, as Gothan says, much depend on leaf fall. Ring and ringless species grow side by side in the tropics, and weak rings may exemplify local conditions or types of structure in higher latitudes. Schenk found the Cretaceous conifers of the Libyan desert without rings, as would often be the case in other forms with persistent leaves. In general, therefore, the rings in essentially modern stem types do indicate temperature—summer and winter, rather than a wet-dry year. But rings are not very pronounced in the xerophylous south Brazilian Araucaria forest (25° to 30° S.), although more marked in the pure stand Chilo-Argentine *Araucaria imbricata* forest of 38° to 40° S. where a snowy and a dry warm season alternate sharply. Both these imposing forests require altitude, and it is believed that the lower slopes of Lake Chelan cutting back into the Cascades from the East in 48° N. are so exactly a physiographic equivalent of the slopes of Lago Aluminé, soil and all, that merely a free planting of seeds is all that is needed to secure a great growth of the *Araucaria* *imbricata* in this yet higher latitude. That means for this forest species an easy sweep poleward of 8° under even present conditions.

Withal, as the dicot stems are so little known, and as the conifers of the Mesozoic were so often leafy and simple of stem, there is nothing in the absence of rings well up within the limits of the present temperate zones which could be taken alone for proof positive of climates much warmer than now—that is, of isothermal variations unaccounted for by the simpler factors of geologic change.

The persistent leaf type has seemed omnipresent in gymnosperms, going back in time, and dicotyl stocks may have been less deciduous in Triassic and Jurassic times than now. But the point had better not be begged. Deciduousness may have been quite as marked in pre-Cretaceous as later forests. Many of the more microphyllous smaller stemmed cycadeoids may have been characteristically deciduous, while there must have been the constant tendency to leaf fall in the dicot lines. And then, too, there were the great circumarctic forests of leaf-shedding ginkgos, and at least up to Liassic times yet other Cordaite relatives of like leaf-fall habit.

It is seen, then, that with relationships amongst the phyla so little known, with an immense preponderance of lowland megaphyllous types in recovered floras, with stem and leaf structure little coordinated, that with the continual uncertainty as to whether the dissociated stems, leaves or fruits are of evergreen or deciduous plants, and with growth rings mainly a feature of modern wood organization, an intense difficulty arises in interpreting the scattered data of fossil rings.

Perhaps the most noteworthy assemblage of later seed plant stems yet brought to light in any country is that from the lower Greensand of the Isle of Wight and nearby, because of the association of both conifers and the oldest found dicotyl stems, described by Marie Stopes. There in N. L. 51° not all the stems have rings, and the more significant is it therefore that Taxoxylon, Podocarpoxylon, Pityoxylon, Protopiceoxylon, Cupressinoxylon, all have fairly marked rings. While the dicot *Aptiana,* which may come from Luccomb Chine in association with *Bennettites Gibsonianus* or Cycadeoidea, and which is structurally held near the tea plant by Janssonius and Moll, and near Vaccinium our northern "blueberry" by Bailey (!) also has the rings. Such facts, such structures, taken with the disposition to view the somewhat earlier Oolitic floras as tropic, teach us all to have a care in using growth rings. Or turn to the southern hemisphere; at Portrerillos on the Trans-Andine I found in rocks presumably of Rhaetic age a characteristic Cupressinoxylon with a well-defined transition from spring to fall wood.

If one were permitted the guess of a lifetime as to climates in the region of the present Isle of Wight in lower Greensand time, in so far as an appeal to the evidence from the plants alone is concerned, conditions might be imagined in which there was a certain nearness to arid belts and some wet winter cold. Nor would any one have needed to travel far to find warm blankets of snow. Just a little earlier the sediments of the Wealden deltas carry Cladophlebis, Sagenopteris, Sphenopteris, Ruffordia, the pseudo tree fern Tempskya, Cycadeoidea and Fittonia, Thuyites and Pinites. But these are not certainly more than warm temperate, and it is left to paleozoologists to speculate on possible habits of migrant pterodactyls or dinosaur winter habits. A little earlier still, in the Purbeck, the Cycadeoideas had reached an extreme of specialization and xerophylly.

It must not be supposed for a moment that there were not from ancient times down forest types that could stretch afar into the world's utmost pole of cold as now. With Arctic lands often entirely wooded and low and the controlling barometric areas variable, it is, then, not needed to drag in tropic climate to any great extent, in accounting for early absence of rings. The ring occurrence is still a dark page, yielding inferences, rather than directly or fully readable climatic data.

TRIASSIC-JURASSIC LEAF CHANGE

Any one who wishes to gain some conception of the foliar change that ended in the Angiospermous forests over and above mere opinion, and probably over and above anything as yet readily gained from tabulation, should for himself pore over the plates of the volumes of Heer's "Flora Fossilis Arctica." And it may be suggested that in so doing there should be at hand the Heer "Bibliographie of Malloizel." This is a little book of 176 pages with an introduction by Zeiller and was published in Stockholm separately and without date. It was really completed by A. G. Nathorst after much labor, a fact of which there is no inkling in the title, and still lacks a tabular study of horizons which some competent student of the dicotyls should yet give.

The publication of these volumes was surely one of the most sensational events in paleobotany and left an impression amongst their readers that the polar areas were long the home of much admixed northern and southern types of vegetation, and that many new types had originated there, later to migrate towards the bases of the north continental areas. At present, such ideas though not entirely abandoned no longer appear simple, being merely mentioned. But the scientific world was left to wonder over problems of celestial mechanics; and Heer himself, without absolutely rejecting the theory of descent, had recourse to ideas of brusque modification of type approaching new creations in the organic world not unlike those of the days of Cuvier.

Now amongst those who have attentively examined the volumes of Heer and then turned to the fossil dicotyl forests further south in a very elemental study of foliar evidence bearing on the ancestry and early climatic environment of the angiosperms are Bailey and Sinnott. They have made a compilation of the *palmate-simple, palmate-lobed, pinnate* and *compound,* Cretaceous and Tertiary species, and they reach the following numerical statement:

	Palmate simple	Palmate lobed	Pinnate	Compound
Cretaceous 908 sp.	15%	15%	59%	10%
Tertiary 877 sp.	26%	7%	55%	12%
Woody Plants 7,014 sp.	9%	4%	65%	21%

The palmate types show an early abundance, and even seem to give way to pinnate vegetation. But here follows a more subtle argument from evidence. It is shown that innumerable floral bracts, calyx lobes, petals and cotyledons, are multi- or very conspicuously tri-lacunar in their bundle supply, and yet accompany pinnate and blade types of leaves which are unilacunar, or nearly so. From all this it is possible to suggest that the old angiosperms were palmate often with three separate bundles at the node, later pulled together into the single midrib strand. And Bailey and Sinnott note that among woody plants uni-lacunar forms now seemingly predominate in the tropics, multi-lacunar and especially palmate lobed leaves in temperate and upland regions. Hence the angiosperms appeared in climates more temperate than tropic, found in much of Mesozoic time only in cool to cold uplands. The view is in reality corollary to earlier theories of polar origins, and is held to favor angiosperm descent from a coniferous palmate rather than a cycadean pinnate stock.

The difficulty of tracing the course of early dicotyl leaf change is so enormous that speculations like the foregoing have the highest value. They, and such alone, lead to more accurate consideration of the elements of the main problem. The actual milestones along the way of angiosperm origin are not seen. But there are other possibilities to take into consideration, as for the greater part merely enumerated here. (1) The picture of change from the break of Cretaceous time until now is one of varied climates with much evolution throughout the angiospermous stock. (2) Parallelism or isolated stocks, or polyphylly means those very long lines that stretched across all Triassic and Jurassic time, ending not in a few but in those many and varied Cretaceous forms. (3) While conifers, cycadeoids and cordaites were distinctively such at the opening of Triassic time, all these groups must have found certain close relationships with near-cordaite, near-conifer and near-cycadeoid types actually leading into angiosperm foliage. Would it be absurd to say that in a larger sense all gave their quota before the break of the Trias, if it is admitted that any one could have done so? (4) While it is true that the various Triassic and Jurassic floras only include, holding in mind a precise chronology, a small fraction of one per cent. of contemporaneous forms for the given point of time, it is unthinkable that paleobotanists have never seen and have never held in hand leaves in the line of descent. There are collections from certain cooler climates like the Argentine Rhaetic, and others from more tropic regions like the Rhaetic (= Keuper) at Lorraine, Virginia; and the suggestion is ever present that many of the leaves recalling Oleandridium, or other ferns, or called Taeniopteris or Nilssonia, or perhaps like the leaves of Williamsoniella or Wielandiella, are merely the foliage of real progenitors of the Cretaceous angiosperms. For it is evident that in the course of dicot evolution leaf change came late or last. (5) The magnolias are a very varied stock far back in the Cretaceous, and probably had pinnate leaves from Triassic times to now. Eames has noted that unique amongst dicotyls so far as observed (but possibly also in Drimys and Trochodendron) the stamens may be added to the Sinnott and Bailey list of floral organs of multilacunar or triadic structure. Three bundles traverse the stamens, and the general floral structure, although without petal or stamen fusion, is elaborate. The magnolia leaves are in agreement decidedly multilacunar.

Only the last of these points as entailing some consideration of bladed types of leaves like Nerium, Sideroxylon, Liriodendropis, Liriodendron and the Magnolias need serve for present purposes of argument. And in reality the entire question as to the trend of lower Mesozoic leaf change resolves itself into whether a sassafras or a magnolia is the more ancient, with the somewhat primitive ancestry restricted; or whether both are old and had very numerous forerunners at the base of the Trias. In either instance it is easy to picture and to admit that there was some change all through the later Jurassic toward simple blades, and *vice-versa* towards palmate leaves. But in the main, and repeating, there is that larger question of palmation and the coniferous ancestry, on the one side, and of blades and a more cycadeous ancestry on the other.

Clearing the way a bit it is observed that unequal rates of change in prevailing groups must always be admitted, and that admittedly the fossil record is so apt to be confined to specialized types and habitats. But starting again from the Flora Fossilis Arctica, it is seen that in the Komé or lower beds of the Greenland series the bladed cycadeoid or Pterophyllum-like form, so very prominent as the old Triassic frond type first to reach world-wide distribution, is still present. And then there is another foliar blade, also old of feature and relationship, the illustrations of which every one might wish to examine for himself. That is the *Nilssonia Johnstruppi* from a little higher up, in the Atané beds which also contain a great Liriodendron bed. Now it is not contended that this latter is not a cycad. But it is a simple type of leaf that has lasted long, and certainly related itself by way of the Ptilophyllums to the extreme or Otozamites types of cycadeoids, culminant over the globe in about Oolitic time. And by the same token there is a relationship to Stangeria of to-day, so far as foliage goes, quite the most primitive of cycads.

Turn, then, to a larger and a greater picture, with the *Nilssonia Johnstruppi* still taken as the turning point, taken as a member, fall where it may, of the Cycadophyta. Perforce any group of plants mainly fossil seems homogeneous or natural only with respect to its foliage. But Tumboa, as a member of the Gnetaleans, if only found fossil could not easily be recognized as the kin of Gnetum, more than once suggested as a possible descendant from some of the peculiar leaf types of the Virginia Rhaetic (= Keuper). And so in the Cycadophyta, the stems and fruits remain for the vast numbers of types included, and for the long time range, mainly inferential. The history of the group simply extends back into contact with the generality of older gymnosperms. The Cycadophytes thus include frond types extending from those with pinnules like the existing *Zamia Wallisii* a foot and a half long by a foot in breadth to very narrow linear and diminutive types. Also the double bifurcate condition in *Cycas Micholitzi* suggests by itself and without even the fossil evidence that could be cited, transition toward Ginkgo leaves. Yet venation may be quite fern-like, or again may run toward a fairly typical net, as in the Jurassic *Dictyozamites*. An interesting intermediate form, cosmopolitan in the later Mesozoic, is the genus *Ctenis*. The Cycadophyta thus range from the bipinnate Bowenia to simple blades, like those of the Cordaites. And such may be sparsely inserted on slender stems, even running into whorls after the manner of dicotyl leaves. The Rhaetic Wielandiella splendidly proves the point, and because of the very broad petiolar almost stipulate lamina may even have had a trilacunar bundle supply.

Much more this simple blade of a form like the ubiquitous *Taeniopteris* has been assigned to the remarkable *Williamsoniella*, unfortunately with some doubt as to absolute correctness. But the gap is at best slight, and it is seen that rapid and easy change from cycadeoid towards the simpler dicotyl blades could have early set in. Amongst the many Jurassic so-called Taeniopterids those with a simple marginal vein have the best chance for dicotyl affinity. It is seen that by a simple and regular invasion of the marginal net with alternant weakening of the pinnate veins the Liriodendropsis type of leaf would arise. And if the stock were somewhat multilacunar there could also be the transition towards sassafras and other palmate types, just as readily as in the reverse direction suggested by Bailey and Sinnott.

An imposing array of transitions can here be suggested. Take the changes exemplified in the magnolias of the Kansas Cretaceous. In *Liriodendron semialatum* the apex is merely obtuse, with a light basal lobing as the name implies. Venation and form may come from the simpler blade of *L. primaevum* Newberry, also present in the Greenland Atané. Then follow *L. Meekii, L. giganteum,* near *L. tulipifera,* which is an old type also. Then, too, there is the *Liriodendron pinnatifidum* with freer lateral lobing, and more retention of bifurcate lateral veins as in some old Ficus types. There is also the pinnatifid *Liriodendron Snowii,* for the removal of which from the magnolias sound reasons fail. What an imposing forest element the magnolias really were down to the Tertiary.

Viewed as a primitive type, or a type surely as old as palmate leaves, the magnolias have both the record and structure in their favor. Taking the young leaf, the venation is for a time even Taeniopteris-like. The mesh develops from the margin inwards, and late. Also, while it could be wished that Bailey and Sinnott had investigated the petiolar supply, it is complex. The bundle alignment is not so very different from that of cycads, while the condition of the stamens above cited is in accord.

If now the point has been scored, if it be admitted that simple leaves are as old as the palmate, that the cycadeoids were an immensely varied frequently deciduous group with real angiosperm contacts, then with other data adduced all these lines are old and in some form traverse all Triassic and Jurassic time. Then, too, it is seen or rather will be admitted that the dicotyl ancestry must be both old and polyphyllic, and it is also easier to grant a somewhat similar status for the ginkgos, and especially the conifers. And if all these things be true a different picture of Triassic vegetation is reached from that so long in view. There is no longer in sight a great group of only tropic cycads compelling the belief that the pines and Araucarias, the leftover Cordaites, the ginkgos, living with them must all be tropic too. Instead there are the mixed forests of generally polyphyletic origin, all the elements of which undergo more or less forward evolution, and thus acquire a prevailing cast. And these varied and manifold types hold their place stubbornly as they react to ecologic factors, each ever striving towards the farthest reach, the remotest habitat, and all alike subject to the fluctuating geologic climates, the changing shore lines, the flow of warmer, then colder waters into the Arctic areas, and that lure of the desert which so specializes plants that they never get back to the moister climates and are cut off. In a word, all those lines from the Triassic down may be thought of as having the power to send some of their members to the ends of the earth, just as surely as the larch and the pine reach the pole of cold now. And for such weighty reasons few of the Triassic-Jurassic seed plant types should ever be thought of as surely tropic, or surely warm temperate without some weightier reason than inference.

Floral Change

A few years ago it was said that the strange thing about the dicotyl advance, as Saporta said, "rapid as universal in its effects," was the flower. And yet when the discussion of natural selection was in its beginnings the Reverend George Henslow had brought out a brilliant book on the "Origin of Floral Structures" in which he ventured the view that, "in a broad sense natural selection seemed obviously true; for Geology had revealed the fact that the world had been peopled over and over again by old forms dying out and new forms coming in; so that although it might account for the extinction of the former, it did not seem capable to account for the origin of the latter." He therefore looked to the environment "as affording a better clue to the source of variations," and mentions as worthy of serious consideration the idea of inherent tendency to higher organization attributed to Naegeli. And Henslow says he returns to 1795 and the "Monde ambiant" of Geoffroy Saint Hilaire.

That is the view taken here. The stems are not in the way, the leaves are not in the way, and the flower is no longer in the way. As far back as the Pennsylvanian there is an as yet not fully understood adumbration of the modern floral type in *Codonotheca* of the Mazon Creek nodules, and in Rhaetic time *Wielandiella,* also lacking in full details of the disk structure, is nevertheless seen to be nothing but a small flower on the Ranalean plan, pertaining to a very simple type of leafy blade of worldwide distribution all through the Trias and Jura. *Williamsoniella* as a less reduced but also microflorous type of the same order of plants only proves the point. While in the Cretaceous the giant flowers of the heavy-stemmed highly specialized cycadeoids at once prove the presence of very ancient lines and show that an essentially angiospermous floral plan held true in the varied types of Mesozoic cycadophytans.

When the flower of *Cycadeoidea ingens* was found the *Williamsoniella* was unknown, and the *Wielandiella* was yet to receive the later study of Nathorst, so that for a time the idea of later floral structure as a result of extreme reductions may have been overemphasized. But at present any such ideas are very much tempered. It is seen that virtually a flower has always been a flower back to the time that many cycads and pines compacted their sporophylls into cones as distinguished from the kind of sporophyll emplacement called a flower in the modern sense. It is better understood that the "sterilization" of which Bower speaks even antedates the leafy terminal cones of the club mosses which are the remote prototypes of modern cones and flowers. And accordingly it becomes very possible that the flowers of the greater cycadeoids, and alike the great cones of Macrozamia weighing seventy to ninety pounds, or the cones of *Pinus Coulteri,* are examples of a secondary gigantism; although this offers no difficulty. The superb flowers of the Himalayan *Magnolia Campbelli,* a foot across (and Magnolia flowers a foot across grow in our own climates, latitude 41°, accompanying leaves 29 inches long), would be no more remarkable, taken as later giant flowers. The triadic staminal bundles of *Liriodendron* noted above are probably general in the magnolias, and show at once the presence of old structure and the ease of variation in sporophyll development. Eames, who has made extended studies of flower histology, independently reaches the view that the magnolia flowers have the tendency to increased size, and he also emphasizes a belief that the varied structure of dicotyl flowers indicates extreme polyphylly.

Put in few words it is then certain that some kind of floral plan essentially ranalean in many small-flowered forms characterized the enormously varied cycadophytans of lower Mesozoic time. And bearing in mind that the recovered fossil record is usually only a fraction of a per cent. of the actual plant life, it is again emphasized that much of the so-called cycadophytan foliage of Triassic and Jurassic times is that of plants near or within the actual line of dicotyl descent. But obviously the larger and more specialized cycadeoids, particularly the Otozamites, Podozamites, Ptilozamites and most typically Williamsonian types, must here be as rigidly excluded from the view as the cone-bearing cycads, and all the post-Permian cone-bearing gymnosperms. It is the deciduous microphyllous, microflorous branched cycadeoids that alone could stand near to, or share in lines of descent; while the geologic-paleontologic fact of a scant and hidden floral record is without significance. No one dares suggest that proportionate to diversity of species in older, somewhat simpler forest alignments, flower growth was not cosmopolitan from the Permian on. It may be suspected, however, that just as the discoveries of cycadeoids have changed all views of cycadeous foliage, so some of the older leafy conifer foliage may pertain to flower-bearing forms. The point made here is that cycadeoids flowers, like the leaves, suggest full adaptability to varied climate, though known in so few forms, and so far in no case outside of Cycadeoidea tied up with stem structure.

ASSUMPTION THAT LARGE LEAFED FERNS ARE TROPIC

So far as can be judged from the approximate 6,200 fern species of to-day, most large-leafed ferns with either simple or compound fronds have always lived in either tropic or moist habitats. But in considering Triassic and Jurassic genera there must be a very large margin of doubt as to the climates indicated by the ferns. Ordinary or true fern foliage being so much like that of the seed ferns, it is inferred that as seed ferns finally disappeared in the Jurassic they were replaced in many forests by simpler fern types like those of to-day with incidental specific variation. Furthermore, as proangiospermous forests merged into the Cretaceous angiosperms, yet another world was opened to the ferns—that of the net-veined canopy. The richer stores of humus and more varied shade conditions meant a final variation into the present fern groups.

Accordingly ferns may be looked upon as far more numerous now than at any preceding time, while precisely the period under consideration must cover a very varied course of fern change just as the record seems to indicate. Inasmuch therefore as the ecology and constitution of the lower Mesozoic fern forest is seen to vary along definite lines, it is affirmable that fern foliage affords merely indirect evidence for tropic conditions of much greater extent than now. Thus what the Dipteris-Matonia group was like in the older and differently constituted forests of the past is a problem beset by many difficulties of chronology, variation in land masses and uncertainty as to temperature tolerance that always steps in in the case of the extinct species of even the fern families. At first it however seems very compelling to learn, as Seward points out, that the Dipteris-Matonia alliance, which includes the singularly handsome lyrate ferns of Rhaetic time extending up to the 60th parallel, is now confined to four genera of the Chinese Malayan region. Especially so when it is added that the hitherto mainly European *Laccopteris münsteri* occurs in the form of fine fertile fronds in the Sonoran upper Trias, and that the curiously characteristic Andriania is found in North America for the first time in the basal Lias of Oaxaca. But it is far easier to cite these examples of change than to tell what they mean in exact climatic terms. For instance, the Schizeaceae, known locally and rarely in New England only from the "curly grass" and "climbing fern," once lived over the northern, but now only persist over the southern world, while the Marattia group, so widely extended as a fossil form, North America excepted, now reaches New Zealand and Cape Horn.

Perhaps I here suggest too much and doubt too superficially. But if I may add a word with myself included I may say that I once stood by a small cool spring within about 200 feet of the very top of a forest-clad, bell-shaped mountain reaching an altitude of some 5,000 feet in southern Oaxaca facing the Pacific. Roundabout there was a little fern garden with many lesser species, clustered round one single noble tree fern of a type I had never noted in the wild before and never had the fortune to find again. It seemed in its surroundings quite the handsomest, stateliest plant I had ever seen. The fine globular trunk had the size of the largest of the cycadeoids, while the fronds of the fertile crown ten feet in length I took to indicate a *Marattia laevis*. Such a fern, but not cycad trunk, and such fronds I would take as certain evidence of tropic monsoon climate though found in fossil beds at the pole. But for many lesser forms polypodium-like, for instance, who shall yet say whether all tropic or not?

RÉSUMÉ

Contradictory as such a dictum might appear, it may be asserted without reservation or doubt that the entire course of fossil plant investigation has reached a point where not only an extreme parallelism and persistence of the greater plant phyla is indicated, but, going back in time a closer and closer contact of phyla. That is why in building up or picturing the long used simile of the "paleontologic tree" discordant views of the lines of descent are invariably reached. The views were never entirely wrong, and yet they are foredoomed to fall very far short of truth. In particular the dominant angiosperms were thought to be very recent in their main evolutionary phase, and therefore sprung from some very primitive or rather generalized and long persistent stirp of now the pines, again the cordaites, or even later the gnetaleans, always hidden away in some far corner of the earth, predestined never to be known.

But a different point of view is reached. There have always appeared, as the ascendant course of plant evolution proceeded, specialized types, generally of giant form and bulking large in the recovered record. Yet the main groups of seed plants have long been discrete and numerous of kind, and in searching for the ancestral forms unequal rates of evolution in the different organs, a point early stressed by Coulter, must be considered. The more does it become necessary to find those types of the pre-Cretaceous horizons with the possibility of restoration; the more is it necessary, in the case of fossils like the continually dissociated stems, leaves and fruits of all lower Mesozoic time especially, to engage in renewed search in regions promising critical evidence. Here may be mentioned the great Liassic-Oölitic series of Mexico, the remarkable Rhaetic laminated shales of Argentina, and perhaps some of the outlying regions of a strong distributional interest like the Mesozoic areas of New Zealand indicating mixed floras with some of the early dicotyls.

And much more, a viewpoint is reached where the lines of descent investigators seek to disclose have practical value, instead of mere academic or philosophic import. To the chronology must be added a bettered genealogy else the paleobotanist is too little guarded against error, too often helpless in any attempt to interpret the climatic data afforded by the lesser, and even some floras of more numerous species. It proves of the utmost interest to know whether a leaf type belongs to a group nearly always tropic or to a more diversified group which had the power to adapt itself to varied climates. It will not do in these climatic discussions to assume that the given type is a gingko, when it may be a pine or a cordiate, a cycad when it is a cycadeoid, or cycadeoid when it may be a full fledged angiosperm. Much of the ancient vegetation, long thought of as only tropic because found in the coal beds or on the limestone-dolomite latitudes and without growth rings, must from ancient days down have had close relationship to or have included types that could grow in the colder climates.

It is perhaps a question on the side as to whether the later dinosaurs could live only in climates like that of the Vero man, Elephas and the last of the saber-tooths or further north in the temperature of the Okeefinokee or of the Dismal swamp region. But perhaps the real point which begins to appear on the paleontologic horizon is not that the climates in which the Dinosaurs lived were worldwide, but that they were of the "asylum" type.

Plant data have been late of accumulation because the localities of critical importance are far flung, the materials often difficult to investigate, and workers few as compared with the vertebrate and invertebrate students with their large resources. Nevertheless a turning point is reached where the plant data are viewed from the possibility of fixed poles and of uniformitarianism. It is fairly evident that the plant alignments of the past may mean but the smallest variations in the general climatic mean for the globe. Probably the largest single contribution that the paleobotany of the future is ever destined to make is a fair determination of the limits or else the disproof of the Knowlton thesis of earlier earth climates dependent on a diminishing factor of interior heat.

SHORTER ARTICLES AND DISCUSSION

CHROMOSOME BEHAVIOR DURING MEIOSIS IN THE POLLEN MOTHER CELLS OF CERTAIN OENOTHERAS

IN recent papers, I have called attention to the peculiar ways in which the chromosomes of Oenothera species behave during meiosis in the pollen mother cells. Six species have been reported on, two of them in detail, and the others in a preliminary way.[1,2,3] Attention has been called particularly to the arrangement of chromosomes in the stage just before the first maturation metaphase, and to the peculiar way in which equal distribution of the chromosomes to the poles is effected. In all the species so far described, the univalent chromosomes during "diakinesis" are found to be so grouped that one or two circles are formed, made up of univalent chromosomes placed end to end. Individual chromosome pairs, such as are characteristic of the cells of most organisms at this period, are present in varying degree, or entirely absent, depending upon the species. Throughout the first maturation metaphase, the circles remain intact, and present a peculiar zigzag appearance, due to the fact that adjacent chromosomes become attached to spindle fibers leading to opposite poles. An equal distribution of chromosomes to the two poles is normally the result of this process.

I have now to report briefly upon five additional species, *Oe. Lamarckiana*, *Oe. rubrinervis*, *Oe. rubricalyx*, *Oe. blandina*, and *Oe. deserens*.

Oe. Lamarckiana: The material upon which these studies were based was obtained from the garden of Professor Geo. H. Shull, at Princeton, New Jersey. Collections were made during the summers of 1923 and 1924. Three strains were examined, all three being derived from plants collected in 1905 by Professor de Vries, at Hilversum, Holland, at the same place at which he obtained his original material. A number of individuals were

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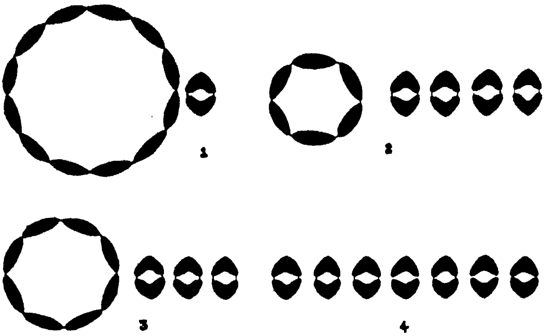
[1] Cleland, R. E., *Amer. Jour. Bot.* 9: 391–413. 1922.

[2] Cleland, R. E., *Bot. Gaz.* LXXVII: 149–170. 1924.

[3] Cleland, R. E., AMER. NAT. LVII: 562–566. 1923.



Figs. 1–4. Diagrams to show chromosome arrangements in late heterotypic prophase. 1, *Oe. Lamarckiana;* 2, *Oe. rubrinervis;* 3, *Oe. rubricalyx;* 4, *Oe. blandina* and *Oe. deserens.*

examined. The three strains have proved to be alike cytologically, as have also the collections from both years.

In "diakinesis," the 14 chromosomes in this species are found to be so grouped that they form a large closed circle made up of 12 univalent chromosomes strung end to end; plus one pair, which at first is linked to the circle, subsequently breaking away from it (Fig. 1). In the heterotypic metaphase, the circle remains intact, and in a large majority of cases the chromosomes that lie adjacent become attached to fibers passing to opposite poles, so that the peculiar zigzag arrangement already described for other species is found here also (Fig. 5). It is interesting to note that this arrangement is the same as that found in *Oe. franciscana sulfurea.*

These results are at variance with those recently reported by Boedyn.[4] According to this author, early diakinesis stages in *Oe. Lamarckiana* show seven double chromosomes, often entirely separate from one another. The single chromosomes making these up may split apart partially during this stage, sometimes to the extent of forming rings, but at all events, remain quite closely paired. With the exception of the one pair always present, however, I have found no pairing of chromosomes in diakinesis. Furthermore, every complete nucleus that I have examined, in which all 14 chromosomes could be clearly seen, has revealed without question the circle of 12.

Oe. rubrinervis: Material of this species was obtained in Professor Shull's garden in 1923. The strain is a selfed line of

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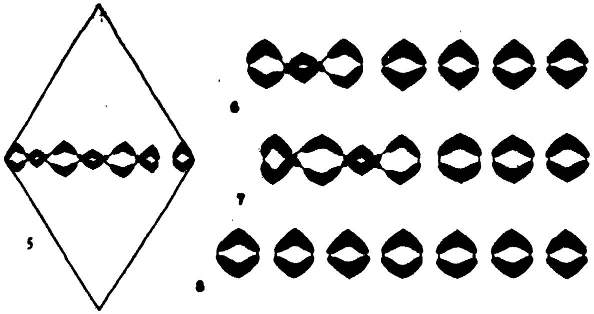
[4] *Zeitschr. f. Zellen u. Gewebelehre* I^2: 265–277. 1924.



FIGS. 5–8. Diagrams to show chromosome arrangements in heterotypic metaphase, side view. Outline of the spindle omitted, except in Fig. 5. 5, *Oe. Lamarckiana;* 6, *Oe. rubrinervis;* 7, *Oe rubricalyx;* 8, *Oe blandina* and *Oe. deserens.*

plants, descended from seed received from Professor de Vries in 1905. My collections were rather small, being confined to one plant, and unfortunately I have been unable this year to obtain material with which to confirm the results gleaned from last year's collection. Hence the results here indicated are to be considered as tentative only. Diakinesis stages in the plant examined showed a closed circle of six chromosomes, with four pairs in the form of rings (Fig. 2). My material was not sufficiently extensive, however, to enable me to determine how these rings were linked together. Beginning with the heterotypic metaphase, the cells of this plant, in many cases, showed a most striking succession of irregularities, the result of which could scarcely be other than the formation of functionless pollen grains; although in some cases, the regular arrangement shown in Fig. 6 was presented. While I am reasonably confident that the suggested arrangement of chromosomes in diakinesis will turn out to be the normal one for the species, I am not at all convinced that these subsequent irregularities are normal, and must wait until I can obtain further material before determining definitely the truth of the matter.

Oe. rubricalyx: Material was obtained from Professor Shull's garden during the summers of 1923 and 1924, from a strain which has bred true for the *rubricalyx* character, and which originated from seed received in 1914 from Sutton and Company —this seed in turn having been derived, by selfing, from a strain sold to that firm in 1912 by Professor R. R. Gates. Collections were made in 1923 from but one plant, but in 1924 they were taken from a number of individuals. Results from the various collections are in perfect agreement. During diakinesis, the chromosomes are arranged into a closed circle of eight univalents, plus three pairs (Fig. 3). The pairs are in the form of rings. During the heterotypic metaphase, the circle remains unbroken, and the zigzag appearance previously described is found here also (Fig. 7).

Oe. blandina and *Oe. deserens:* Extensive collections of these species were made from plants grown at the University of Pennsylvania, from seed sent me in 1923 by Professor de Vries. Quite a number of individuals of both species were studied cytologically. Both show in diakinesis a perfect pairing of chromosomes. No large circles are present (Fig. 4). The pairs are ordinarily in the form of rings. In the heterotypic metaphase, the pairs line up in a perfectly regular manner, and the chromosomes are separated as they are in most plants and animals (Fig. 8). These two species are the first that I have examined that show 100 per cent. pairing. The significance of this fact I shall try to point out in a subsequent paper.

The species that I have mentioned in this paper are closely related to one another. *Oe. rubrinervis* is a "half mutant" from *Oe. Lamarckiana. Oe. deserens* makes up approximately one third of every culture from selfed *rubrinervis. Oe. rubricalyx* arose from a culture of *rubrinervis* in 1907.[5] *Oe. blandina* arose in 1908 in the 4th generation of the cross *lata* × *semilata,* and according to de Vries[6] represents the union of two *velutina* gametes, derived from *Oe. Lamarckiana,* but lacking lethals, as well as being slightly modified in other ways.

The species or varieties that I have studied cytologically now number eleven. Two show perfect pairing (*Oe. blandina* and *Oe. deserens*), one has a circle of four chromosomes, and five pairs (*Oe. franciscana*) and one has a circle of five, and five pairs, with frequent modifications (*Oe. oblonga*); one has a circle of six, and four pairs (*Oe. rubrinervis*), one a circle of eight, and three pairs (*Oe. rubricalyx*), two a circle of 12 and one pair (*Oe. Lamarckiana,* and *Oe. franciscana sulfurea*), and three of them have no pairing at all (*Oe. biennis, Oe. biennis sulfurea* and *Oe. muricata*). Although we have here a very striking variety in chromosome arrangement in diakinesis, the uniformity with which an arrangement is maintained in a given species is just as striking; as is the uniform presence of the zigzag arrangement of chromosomes during heterotypic metaphase in all species possessing large closed circles.

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[5] Gates, R. R. "The Mutation Factor in Evolution," London, 1915, p. 103.

[6] *Biol. Zentralbl.* 43^2: 213–224. 1923.

A discussion of the possible relation of these facts to the problems of linkage, crossing-over and the origin of "mutants" will appear shortly, and detailed accounts of the various species will be forthcoming as rapidly as time permits.

It is a pleasure to acknowledge my indebtedness in connection with these studies to Professor Geo. H. Shull, Professor Hugo de Vries and to Professor Rodney H. True.

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A NEW LEG MUTANT IN DROSOPHILA MELANOGASTER

DURING the experiment of the author with Eyeless, a number of flies appeared with shortened and thickened tarsal joints. It seems best to give this character a name in order to make its discussion easier. So large a number of mutants have been described in *Drosophila melanogaster* that it is rather difficult to choose a name that will not conflict with one previously used. In this paper we shall refer to this new mutant as Thickened.

A pure stock of Thickened was prepared. A cross with Star Dichaete showed that the gene for Thickened was recessive and located in the second chromosome. One culture was arranged to give crossing-over from Star, and this showed a cross-over of 35 per cent., indicating that the gene was near Black. A Black-thickened-vestigial stock was therefore prepared.

In the preparation of this stock Black-purple-vestigial was used. Only cultures prepared from double cross-overs, that is, those that had split out purple, gave Black-thickened-vestigial flies. This of course indicated that Thickened is located between Black and Vestigial and near Purple.

Black-thickened-vestigial flies were crossed to wild and the F_1 females back-crossed to Black-thickened-vestigial. The results are summarized in the table.

TABLE SHOWING THE CROSSING-OVER BETWEEN BLACK, THICKENED AND VESTIGIAL

		Black and Thickened		Black and Vestigial		Thickened and Vestigial	
Culture No.	No. of individ.	No. of cross-overs	Per cent.	No. of cross-overs	Per cent.	No. of cross-overs	Per cent.
1	196	13	6.6	35	17.8	26	13.3
2	210	12	5.7	13	6.2	1	0.5
3	247	15	6.1	31	12.6	16	6.5
4	181	12	6.6	33	18.2	25	13.7

It is evident that a cross-over modifier has been involved in culture 2 and probably in culture 3. Sturtevant (1919) has shown that such modifiers exist. This one, however, has not affected the crossing-over between Black and Thickened, so the average of the four cultures should give something close to the correct value. This is 6.2 per cent. The average cross-over percentage between Thickened and Vestigial in cultures 1 and 4 is 13.5. Bridges and Morgan (1919) give 6.2 as the cross-over percentage between Black and Purple, 11.8 per cent. between Purple and Vestigial, and 17.8 per cent. between Black and Vestigial. Thickened is therefore very near to Purple.

In viability Thickened seems to be fair, being fully equal, if not better than Vestigial in this respect.

Stocks of this mutant can be obtained from Dr. W. M. Barrows, Department of Zoology, Ohio State University.

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PROTOPLASMIC SYSTEMS AND GENETIC CONTINUITY[1]

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THE cell is a complex heterogeneous system that arises only by division of a preexisting system of the same general type, and with certain minor qualifications the same is true of each of its two primary components, nucleus and cytosome—each of these is itself a complex system that arises by division of a preexisting system. Cells may thus reproduce themselves for a period that has no assignable limits. We long since learned, accordingly, to think of cell-division as the central phenomenon in heredity, yet prolonged research has left us uncertain concerning many of its problems.

So far as nuclear division (karyokinesis) is concerned our analysis is now well advanced. The progress of investigation, both cytological and genetical, long since demonstrated that karyokinesis is a meristic process involving a spinning out of the nuclear substance into long threads (spireme) and their longitudinal fission, by which is accomplished a precise doubling and equal distribution of all the essential components of the system (chromosomes, chromomeres, genes, etc.). Our acquaintance with division of the cytoplasmic system (cytokinesis) is, on the other hand, in a very backward state. Superficially regarded, the process offers the general aspect of a simple mass-division; and as such it has rather generally been regarded. Cytokinesis shows in fact nothing exactly comparable to the spinning out of the nuclear substance to form dividing spireme-threads and chromosomes. Nevertheless, the researches of recent years have prominently raised the question whether cytokinesis should not, like karyokinesis, be regarded as meristic in type, even though it be less exact in operation.

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[1] In substance a lecture given at the Marine Biological Laboratory, Woods Hole, July 10, 1925.

It has been rather generally held, probably correctly, that many formed components of the cytosome (granules, fibrillae, etc.) arise epigenetically (*"de novo"*) in the hyaloplasm without discoverable direct connection with their predecessors in earlier generations; though the possibility has always remained that some of them might grow from preexisting smaller bodies lying below the horizon of microscopical vision.[2] I will not discuss this possibility here nor enter into speculations concerning a supposed ultra-microscopical metastructure of protoplasm (micromerism, pangenesis, etc.). For the present we are more interested in questions raised by direct observations on the visible formed cytoplasmic components, in particular the central bodies, plastids, chondriosomes and Golgi-bodies. Both plastids and centrioles, admittedly, are capable of self-perpetuation by growth and division, though it is not certain that plastids have no other mode of origin, and it now seems to have been finally demonstrated that under certain conditions centrioles may arise *de novo*. In respect to the chondriosomes and Golgi-bodies the status of the question is much more doubtful, though it is known that both may at times break up into smaller bodies by some sort of division or fragmentation. Both these kinds of bodies are of especial interest, for many reasons, in connection with the problem of genetic continuity in cell-systems. With the advance of research the probability grows that chondriosomes are omnipresent in the cells of both animals and plants; and, in the case of animals at least, the same may be said of the Golgi-bodies. It is certain that each of these kinds of bodies consists of a specific material capable of great if not unlimited growth. It has been made clear that in a large class of cases both are received as such from the mother-cell during mitosis (chondriokinesis, dictyokinesis). What does not yet clearly appear is whether either or both are self-propagating in the same sense as chromosomes, plastids or centrioles.

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[2] Altmann, Heidenhain. See Wilson, 1923, 1925.

In addition to the foregoing facts we may emphasize the presence of chondriosomes and Golgi-bodies in relatively undifferentiated cells having extremely little cytoplasm. For example, in the early germ-cells of scorpions (spermatogonia, spermatocytes) the cytosomes are in certain stages so small that the tissue gives the appearance of a syncytium (though cell-boundaries are in fact present), the cytoplasm being reduced to hardly more than thin films between the large and crowded nuclei; yet both the bodies in question are still conspicuously present (Fig. 1). Here it would almost seem that the cytosome contains little more of the ground-substance or hyaloplasm than suffices to carry forward the chondriosomes and Golgi-bodies (no doubt also the centrioles) to a later period when they play their part in the growth and differentiation of the germ-cells. Such facts indicate that these bodies must be of great importance for the operation and maintenance of the cell-system, even though their physiological rôle has not yet been precisely determined.

There is much in the literature to support the conclusion that both chondriosomes and Golgi-bodies play an important part in the production of other formed components of the cytosome. Recent researches have produced strong evidence that the Golgi-bodies are immediately concerned in the formation of the secretory granules, and the possibility is thus opened that they may be concerned in the production of the organic enzymes generally.[3] There is evidence tending to connect them with the formation of excretory granules and of storage-granules (yolk, fat); and it is certain that they form the source of the acrosome of the animal sperm.[4] Concerning the rôle of the chondriosomes the case is still widely open. It seems, however, very probable that they are

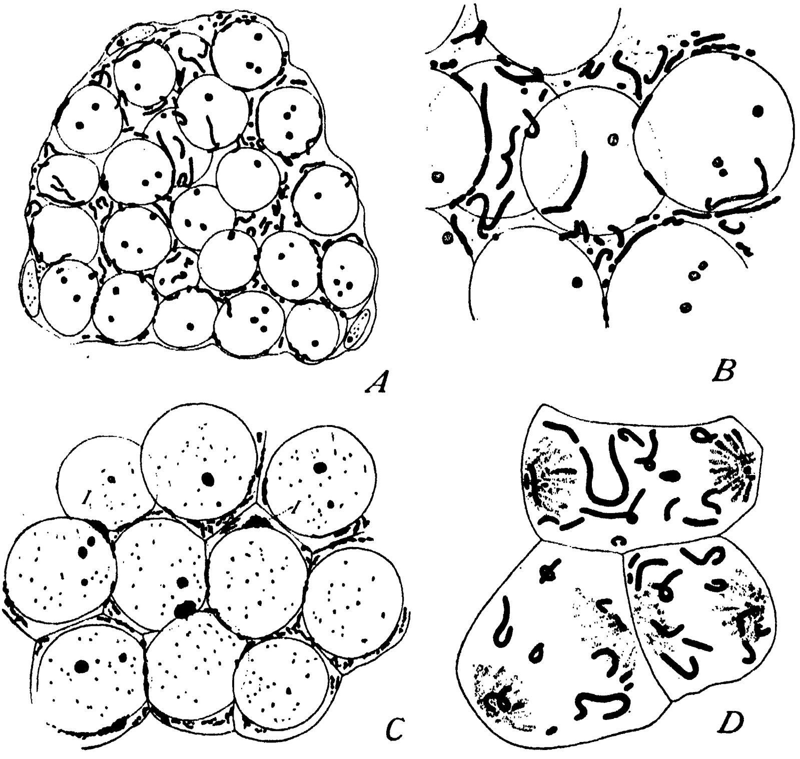

FIG. 1. Spermatogonia (and spermatocytes?) of *Opisthacanthus* (A × 1000 +; B – D × 2000 +)

A, section through complete cyst of large spermatogonia (Benda method) with closely crowded nuclei and chondriosomes (black); B, detail from same, more enlarged; C, smaller spermatogonia (perhaps young spermatocytes), Flemming iron-haematoxylin, showing cell-boundaries, chondriosomes (damaged by the fixation) and idiozomes, I (Golgi-apparatus); D, anaphases of mitosis, large spermatogonia (Benda method), sorting out of whole chondriosomes.

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[3] See Nassonov (1923, 1924), Bowen (1924a).

[4] See Bowen (1924b), with literature.

centers of specific chemical action (Regaud), perhaps effected by surface-action (Cowdry), a view which fits well with the earlier conclusion of Altmann, Meves, Duesberg, Guilliermond and others that they may be in some manner the source of many other formed bodies, including the various forms of plastids and certain types of granules and fibrillae. This conclusion, it is true, has encountered considerable opposition, and opinion concerning it is still unsettled; nevertheless I believe that it deserves the most careful further consideration. But whatever be the issue of this particular question the known facts sufficiently emphasize the importance of both chondriosomes and Golgi-bodies for every inquiry into the nature and perpetuation of cytoplasmic systems.

The cytological aspects of this subject will here be discussed briefly in the light of studies on the chondriosomes (to some extent also on the Golgi-bodies) of scorpions, on which I have long been engaged. I have examined the history of these bodies in the male germ-line of four genera, namely, *Centrurus* (two species) *Opisthacanthus, Vejovis* (two species) and *Hadrurus*. These forms offer material of exceptional interest for the problem because, as heretofore reported (Wilson, 1916), they show two widely different, and at first sight irreconcilable, types of chondriosome-behavior in the spermatocyte-divisions. One of these has thus far been found only in *Centrurus*. Here the chondriosomes, originally small and scattered, enlarge and finally fuse into a single large ring-shaped body which is exactly divided in each spermatocyte-mitosis and the chondriosome-material is thus equally allotted to all the sperms. In the contrasting type, found in the three other genera mentioned,[5] the chondriosomes remain always separate and enlarge to form much larger rounded chondriospheres of approximately constant number (in *Opisthacanthus,* ± 24). These bodies undergo no division but remain scattered through the cytosome, and in the course of each spermatocyte-division are sorted out whole into two nearly equal groups (Figs. 2, 3). The second spermatocytes thus receive each about twelve chondriosomes and the spermatids six; but this process is demonstrably inexact.[6] All theory aside, it is remarkable that bodies so closely similar in their earlier stages, and identical in destiny (formation of the envelopes of the sperm-tail), should differ so radically in mode of distribution to the sperm-forming cells.

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[5] This type was earlier found by Sokoloff (1913) in *Euscorpius*, more lately by Gatenby and Bhattacharaya (1925) in *Palamnaeus*.

In the hope of throwing some light on this puzzle I have attempted to trace the history of the chondriosomes in earlier stages of the germ-line, in so far as they are shown in the mature testis, and incidentally have made some observations on the Golgi-apparatus. In spite of some difficulties this material offers many advantages for such a study. After the earliest stages the chondriosomes are relatively large and few and stain brilliantly by the Benda method and others. The mature gonad contains large numbers of separate cysts of all ages, each composed of numerous cells at nearly the same stage of development. In each cyst the chondriosomes are of the same general type, though cysts of different age differ materially in this respect. Since an enormous multiplication of cells takes place during the growth of the testis, and since numerous chondriosomes are present at every stage, there must be a corresponding multiplication of these bodies. How does this take place?

In respect to this question, so important for our general conceptions of cytoplasmic systems, a conflict of opinion now exists. It is generally, but not unanimously, agreed that the chondriosomes are of strictly cytoplasmic origin. It is certain, as above stated, that in cell-division many of them pass from mother-cell to daughter-cell. Altmann, Benda, Meves, Duesberg, Guilliermond and others have considered them as bodies that are genetically continuous from generation to generation, a conclusion summed up in Duesberg's statement that "every chondriosome arises from a preexisting chondriosome." (1910, p. 122.) This does not necessarily mean that chondriosomes, like plastids or chromosomes, are definitely organized and self-perpetuating bodies, as originally maintained by Altmann. A cautious attitude in respect to this interpretation was taken by Benda, Meves and Duesberg; but it has been to a certain extent implied in some of the writings of both their followers and their critics. It has been supported by some important researches, prominent among them those of Fauré-Fremiet (1910) on cell-division and chondriokinesis in ciliates, and those which have indicated the identity of plastids (bodies which undoubtedly multiply by growth and division) with chondriosomes or their origin from them. (See Meves, 1917.) A widely different conclusion is reached by Schreiner (1916) whose results demand the most careful attention. This excellent observer found that during the prophases of mitosis in epithelial cells of *Myxine* the long thread-like chondriosomes fragment into much smaller bodies, which are then sorted out whole into the daughter-cells; but he also reached the remarkable conclusion that their numbers are periodically reinforced by new formation from extruded fragments of *nucleoli*. This result, almost alone among those of modern observers, is in principle akin to the earlier ones of Goldschmidt (1904) and his followers, now generally abandoned. A few observers, finally, have concluded that chondriosomes may arise *de novo* in the cytoplasmic substance.

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[6] Careful counts of 500 spermatids in *Opisthacanthus* showed in round numbers 76 per cent. with six chondriosomes, 17 per cent. with five, and 7 per cent. with seven. In *Vejovis* the variation is somewhat greater.

Without attempting here a critical discussion of these divergent views I will briefly summarize the main conclusions concerning them indicated by my studies on the germ-cells of scorpions, as follows:

(1) In the spermatogonia and spermatocytes of these animals not the least evidence has been found that nucleoli or nucleolar fragments are at any stage extruded

from the nuclei, or that chondriosomes have such an origin. All the evidence indicates that at every stage the chondriosomes are strictly cytoplasmic structures.

(2) That chondriosomes may arise *de novo* (i.e., without direct connection with preexisting chondriosomes) has been accepted by a considerable number of observers, both from studies on living material,[7] and on sections;[8] and the possibility of such an occurrence is admitted even by Duesberg, a leading advocate of the genetic continuity of chondriosomes (see 1915, p. 62). By neither method, admittedly, is it easy to obtain crucial evidence for or against this possibility; and no conclusions can yet be stated otherwise than tentatively. Nevertheless, the facts observed in cells of the germ-line in scorpions point to the conclusion that in this case, at least, chondriosomes always arise from preexisting ones. Did they form *de novo* we should expect to find among them all gradations in size down to the limit of vision. But in this material such is not the case. In the spermatogonia (Figs. 1, 3, F) they appear in the form of threads and rods, of nearly uniform diameter and with rounded ends, which graduate down to spheroidal mitochondria of nearly this same diameter, or a little less, but do not in general pass much below this limit. Here and there, it is true, still smaller granules of doubtful nature may be seen; but their number seems far too small to form an adequate source of the numerous larger chondriosomes.

(3) On the other hand, in every cyst of spermatogonial anaphases and telophases may be seen the demonstration that the chondriosomes of the mother-cell are being sorted out into two approximately equal groups that pass into the daughter-cells.

(4) The sorting-out process is not accompanied by any general or regular process of division. Especially in certain cysts of larger spermatogonia this is evident beyond the possibility of doubt (Fig. 1, D). The chondrio-

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[7] Chambers (1915), Lewis and Lewis (1915).

[8] See Schreiner (*ante*), Luna (1913).

somes—here relatively very large, but showing all gradations from long rods through shorter ones down to spheroidal bodies—may with perfect clearness be seen passing to the poles without the least sign of division or fragmentation.

(5) There is nevertheless almost conclusive evidence that the chondriosomes increase in number by a variable process of fragmentation, which in case of the shorter rods often appears as an equal or subequal fission closely similar to that described in the ciliates by Fauré-Fremiet, both *in vivo* and in sections. All stages of this process may be seen, including various degrees of constriction (dumbbells) leading up to separate paired spheroids. In these animals, however, the process is not synchronized with nuclear division. Such figures are seen here and there during mitosis along with many simple rods passing undivided to the poles (Fig. 3 C, D); but they are also of common occurrence in the interphases (Figs. 1, A, B; 3, E). In case of the longer rods and threads the process is much less regular and rarely is seen during mitosis. In the interphases the threads are often single, without sign of fragmentation; but often, too, they show all stages of what I can only interpret as a fragmentation into shorter rods or even spheroidal bodies. The process here seems to be quite irregular; but occasionally the products are seen in regular alignment and in close series (Figs. 1, A, B, D; 3, E). Processes of this type have often been described, both in fixed preparations and *in vivo*;[9] but their significance has not, I think, been sufficiently recognized. Here again, admittedly, it is difficult to reach a certain conclusion from the study of fixed material alone. Nevertheless, in view of all the facts, it seems highly probable that the longer rods and threads arise by the terminal growth of shorter ones which themselves arise by the fragmentation (in some cases an equal division) of longer ones as above described. In a certain

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[9] See, for instance, Schreiner (1916), Lewis and Lewis (1915), Lewis and Robertson (1916).

sense, therefore, the chondriosomes are self-perpetuating; but this should not be taken to mean that they are autonomous bodies as such. It is, I think, more probable that they are variable, plastic and polymorphic forms assumed by a specific substance which is *per se* capable of extended and perhaps unlimited growth; which arises from a preexisting substance of the same kind, and is genetically continuous in successive generations of cells; but which is not definitely or permanently organized to form individualized structural units.

The distinction thus drawn is, I think, more than a merely verbal one and involves broader questions. To what extent may such a conception apply to other components of the cytoplasmic system? Nearly all recent studies indicate that it may be applicable to the Golgi-bodies, though the facts are here less fully known. The observations of Deineka, Hirschler, Nassonov, Gatenby, Bowen, Cowdry and others have shown that the Golgi-material too is capable of extensive growth; that it is to a considerable extent polymorphic; that at certain stages the Golgi-bodies fragment; that in cell-division the substance of these bodies is passed on in the form of dictyosomes from mother-cell to daughter-cell. With all this my observations on the germ-cells of scorpions are in agreement, though they are admittedly inadequate and must be taken with a certain reserve because based on an unsuitable technique.[10] Nevertheless, the phenomena shown in this material are of such interest as to merit brief mention here. The Golgi-bodies seem to be present at every stage from the spermatids backwards to the earlier generations of spermatogonia, in which the cytoplasm is so scanty as to form only a small fraction of the nuclear volume. During the whole growth-period of the spermatocytes (apparently also in the spermatogonia) the Golgi-apparatus appears in the form of a rounded but somewhat irregular idiozome closely applied to the nucleus, and in later stages is clearly seen to consist peripherally of rodlets or plate-like bodies stained deep black by the haematoxylin and having no connection with the chondriosomes. In the first spermatocyte-division its behavior is of unusual and interesting type. During the middle prophases the idiozome always draws out along the nuclear membrane, and in some cases its main mass is clearly seen dividing into two parts (Fig. 2, B, C), though there are many indications that it may also fragment into a larger number of smaller pieces. I have been unable to follow its later history completely; but in the later prophases what I believe to be the main part of the Golgi-substance is seen approaching the spindle-poles in the form of two fairly definite masses composed of deeply staining rod-like bodies (Fig. 2, D). A varying number of smaller bodies of similar type are however also often seen scattered elsewhere in the cytoplasm. At full metaphase the two masses are always closely applied to the spindle-poles, over which they fit like caps, so that the centrioles, previously clearly visible, are now lost to view (Fig. 2, E, F). In this position the two masses pass into the respective daughter-cells, and during the interphase are seen lying near the nucleus as a plate-like mass (Fig. 3, A). In the second mitosis quite similar masses are again seen at the poles (Fig. 3, B) passing thence into the spermatids, where, as usual, they take part in forming the acrosome. A similar process of dictyokinesis seems to take place in the spermatogonial divisions, though this is less certain owing to the difficulty of distinguishing here between the Golgi-bodies and the chondriosomes (Fig. 3, F).

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[10] Material fixed especially for the Golgi-bodies (Kopsch method and modifications) failed to give satisfactory results. The following account is based on gonads fixed in strong Flemming's fluid and stained with iron-haematoxylin. In this treatment the Golgi-material has no doubt suffered some damage; but I believe the identification to be correct, and the pictures are in most respects very clear.

These observations indicate a mode of dictyokinesis akin to that described by Nassonov (1922) in *Triton* and by Ludford and Gatenby (1921) in mollusks and mammals, but showing an even more definite tendency of the

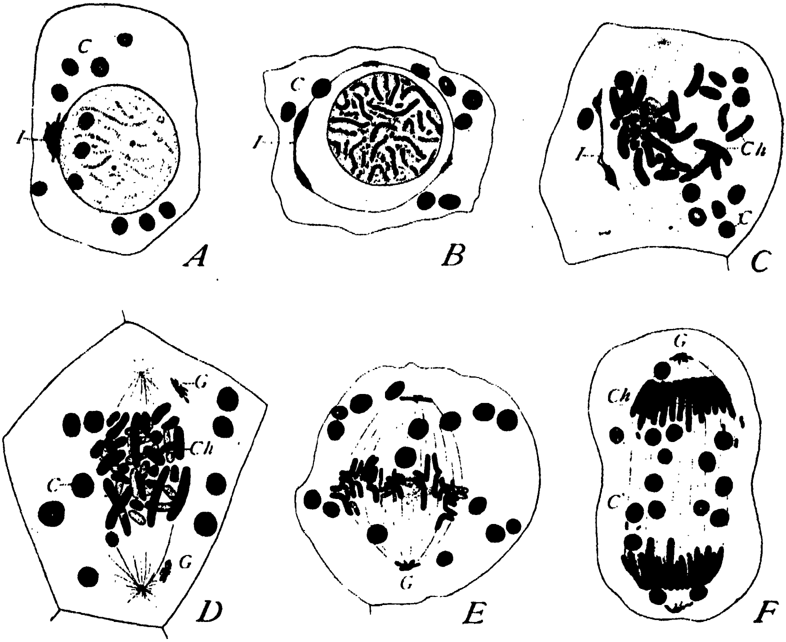

FIG. 2. Primary spermatocytes of *Opisthacanthus* (Flemming-iron-haematoxylin), chondriospheres (C), idiozome and Golgi-apparatus (I, G) and chromosomes (*ch.* × 2000 +).

A, middle growth-period, with idiozome surrounded by Golgi-bodies, chondriospheres; B, early first-division prophase, contraction-figure, idiozome dividing; C, later prophase, idiozome dividing, centrioles, chondriospheres, chromosomes; D, late prophase, Golgi-masses, passing to the poles; E, first metaphase, Golgi-masses covering the centrioles; F, anaphase of same, showing Golgi-masses at poles and a few scattered Golgi-bodies (?).

dictyosomes to mass at the spindle-poles. These phenomena emphasize the conclusion of Bowen (1920) that chondriokinesis and dictyokinesis involve a relation between the cytoplasmic formed bodies and the division-centers that means more than a merely fortuitous association. Whether the Golgi-material may form in the cytosome *de novo* is an open question on which the attention of cytologists is now closely centered. The possibility of such an origin has been admitted by some of the most recent observers;[11] but it seems at least equally possible that the reconstruction of the Golgi-apparatus that follows the breakdown occurring after secretory activity, or in the course of dictyokinesis, may always take its point of departure in preëxisting Golgi-material. Aside from this doubtful point both the morphology of this apparatus and its history in cell-division suggest a conclusion analogous to that above outlined in respect to the chondriosomes. The facts indicate that the Golgi-bodies, too, consist of a specific and self-perpetuating stuff that is genetically continuous from one cell-generation to another, but is morphologically polymorphic and not very definitely organized into self-perpetuating structural units. It is an interesting question whether this stuff,

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[11] See Bramble (1925), Ludford (1925).

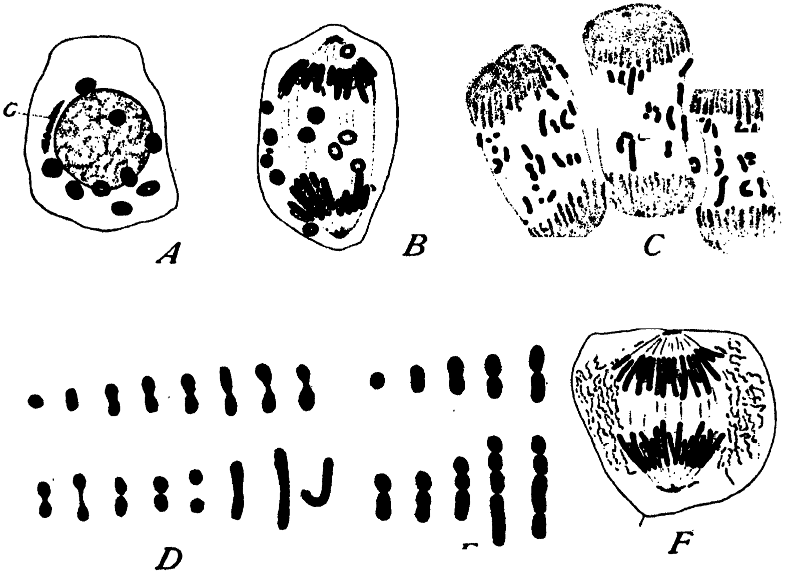

Fig. 3. Various stages of spermatogenesis, *Opisthacanthus* (A, B, C, F, ×2000+, Flemming-haematoxylin, C-E, Benda method). D and E enlarged about twice as much as the other figures.*

A, interkinesis following first division, Golgi-mass at G; B, second division anaphase (*Cf.* Fig. 2E); C, anaphases of small spermatogonia, a few of the chondriosome-rods constricting; D, various forms of chondriosomes, more enlarged, from anaphases like the last; E, chondriosomes (at the same enlargement) from interkinesis of the larger spermatogonia (as shown in Fig. 1 A, B); F, very early spermatogonial anaphase (from rosette-cyst), with thread-like chondrioconts, Golgi-bodies (?) at the poles.

* In Fig. 3 C. the chondriosomes are represented slightly too large.

in each case, is a single and homogeneous substance or may consist of specifically different components; and the question may even be raised whether chondriosomes and Golgi-bodies (like chromosomes) may differ qualitatively among themselves. Such a possibility, certainly, should not be lost sight of, especially in case of the chondriosomes, because of their possible rôle in differentiation.

The foregoing considerations involve important issues relating to the nature and perpetuation of protoplasmic systems generally. Modern cytologists (and *a fortiori* cell-physiologists) have looked askance at particulate or pangenistic views of cell-structure in so far as these have involved the conception of protoplasm as a composite built up from protomeres or self-propagating units, whether visible or ultra-microscopical. Large concessions to such conceptions have been forced by researches on the nucleus, both cytological and genetical; but so far as the cytosome is concerned most recent researches, the present one included, have pointed in a different direction. The possibility nevertheless remains that cytoplasmic systems generally may include many different stuffs that are self-perpetuating by some kind of autocatalytic process.[12] We may venture the further surmise that such stuffs may undergo individuation in various degrees to produce visible formed bodies which in their fullest development became definitely organized as such and capable of self-perpetuation. Centrioles, for example, undoubtedly may perpetuate themselves by growth and division; yet it has been made extremely probable that under certain conditions they may also be formed *de novo.*[13] It has been held that the same may be true of the plastids of plant cells, though this seems never to have been definitely proved.[14] In case of the chondriosomes it would seem (if the results of Fauré-Fremiet are well founded) that the fragmentation of chondriosomes, though commonly irregular, has in some cases culminated in a regular process of fission, synchronized with mitosis.

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[12] See Troland (1917).

[13] *Cf.* Wilson (1901, 1925), Chambers (1921). See also experimental studies by Fry and by Tharaldsen now in press.

[14] See Harper (1919).

Admittedly, the foregoing suggestions involve a somewhat preformistic conception of cytoplasmic systems; but it is one that differs widely, I think, from the intracellular pangenesis of Weissmann, Altmann and similar micromeristic hypotheses of other earlier writers. Should it prove well founded it might offer a more acceptable alternative to those speculative constructions—one that is more in accordance with modern views of protoplasm and comes more closely into touch with the problem of development. Experimental studies in embryology have clearly demonstrated the correctness of the early conclusion of cellular embryologists that the cytoplasm of the unsegmented egg contains specifically different, but not visibly formed, materials that are segregated in definite manner by the process of cleavage.[15] Whether any or many of these are self-perpetuating is a question of far-reaching interest alike to the embryologist, the cytologist and the geneticist, and hardly less so to the biophysicist and the biochemist.

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[15] See Wilson (1925).

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PARASITISM AMONG THE HELMINTHS[1]

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INTRODUCTION

IN considering the parasitic helminths and their habits one is reminded of the popular presentation of the subject by P. J. van Beneden in "Animal Parasites and Messmates," which was of appealing interest to the naturalist of five decades ago. Since that time, however, the knowledge in this field has increased tremendously, although regrettably a considerable portion of the data deals with systematic classification and description of species. However, Schauinsland, Leuckart, Looss and others, who worked on the life-histories of various forms, introduced the experimental method into the study of the groups and became the forerunners of the helminthology of the present day. The important rôle which many of the helminth species of man have been found to play in human disease in tropical and oriental countries has within recent times stressed that phase of the subject, perhaps to the disadvantage of the problem of parasitism among helminths as a physiological group, since it shifted the interest from the field of pure biology to that of an applied field. It is worth while, therefore, to pause for a few moments, and to consider the subject of parasitism among helminths as a whole, in the broader, more theoretical, but at the same time more fundamental sense, in order that one may reconsider the problems of a half century ago and may recast and reformulate theories on the basis of the data now in hand and the prospects immediately in view.

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[1] Contribution from the department of medical zoology, School of Hygiene and Public Health, Johns Hopkins University, and the parasitology laboratory, Peking Union Medical College.

For practical purposes one may divide the parasitic helminths into two groups, namely, those belonging to the Platyhelminthes or flatworms, including Trematodes and Cestodes, and those belonging to the Nemathelminthes, or roundworms. Although it is not my purpose to discuss the classification of these groups, I shall present my subject as regards the trematodes, cestodes and Nemathelminthes first under separate headings, and then proceed to a general consideration of the phenomena attendant on parasitic adaptations among the helminths as a whole.

THE TREMATODES

The primitive development in the Trematodes was a direct one, with the larva as an important stage in development. (See text figure A). In the case of the ectoparasitic flukes, this type of development has not been materially or fundamentally altered, the only modification being that of a differentiation of the posterior part of the body of the larva into a holdfast organ for the purpose of attachment of the worm to the external tissues of the host (Plate I, fig. 2b). Thus the hermaphroditic adult produces ova in which the embryos develop to a certain stage when they escape from the egg shell, find a place of attachment for nourishment on the gills or in the bladder of the new host and, in turn, develop to adulthood.

In the case of the other group of trematodes, an endoparasitic existence must have become a fixed and obligatory condition early in their phylogenetic career. All lines of careful and weighed evidence point to the probability that the first host to which they became adapted was the mollusc. Some of the reasons for this belief may be stated as follows. (1) The mollusc is an invertebrate host, mainly adapted to an aquatic or moist habitat such as that favorable for the existence not only of the primitive trematode but of the present free-living relatives of the group (the Turbellaria). (2) The mollusc is a cold-blooded animal with body fluids and tissues not essentially different in tonicity and nourishment content from those of the simple platode. (3) The first relationship of the trematode to the mollusc was undoubtedly an ectoparasitic one on the organs in the body cavity, particularly the kidney. The habitat of Aspidogaster and other Aspidocotylea is essentially this place to-day. As far as is known, the larva of this group reaches this position soon after its escape from the egg and here grows to adulthood (Plate I, fig. 2 c). (4) With the development of salivary glands for digesting of host tissues, a condition actually obtaining in certain aspidobothrids (l. c. *sg*) and elaborated very considerably in the digenetic trematodes, penetration of the molluscan host tissues was effected, where a source of nourishment as well as a comfortable place of residence was provided in the lymph spaces which bathe all the essential organs of the body. Gradually, the flukes became localized in the interhepatic lymph spaces and those surrounding the hermaphroditic organs of the mollusc, where a place of nourishment *par excellence* was provided.

Between this essentially simple primitive condition of endoparasitism and that of the complex situation in the majority of digenetic trematodes, where one or more vertebrate hosts are involved, there is a very wide gap, which is represented in fact by various conditions of complexity, with modifications, elaborations, "short-cuts" and adaptations differing almost in every species which has come under investigation.

For convenience we may refer to the various stages in the life-cycle of the digenetic trematode as follows:

(1) The *miracidium,* or larva hatched from the fertilized egg (Plate I, fig. 1b).

(2) The *parthenita* (or parthenitic generation).[2] Parthenitae are those generations in the molluscan host in which reproduction is parthenogenetic and viviparous (Plate I, figs. 1c and 2d).

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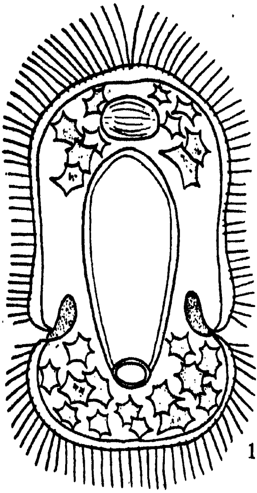
[2] The term "parthenita," now recognized by trematode investigators, was originally advocated by Ssinitzin (1911), and introduced into the western literature by the present writer in 1918.



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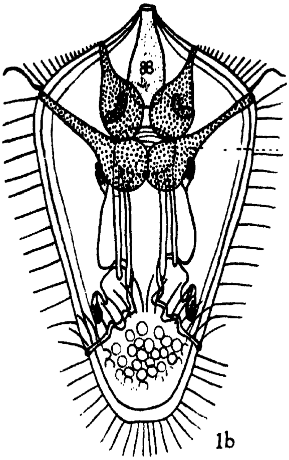

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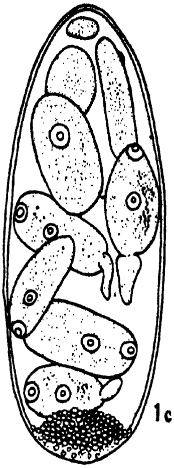

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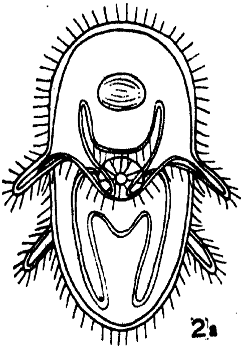

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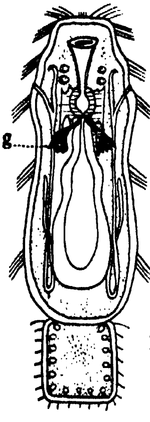

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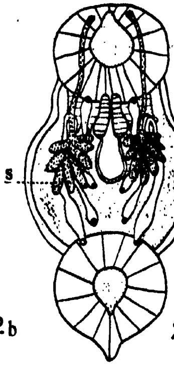

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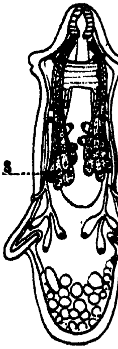

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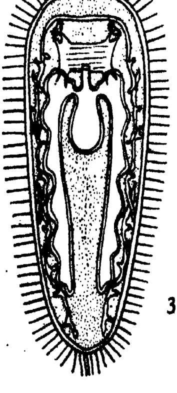

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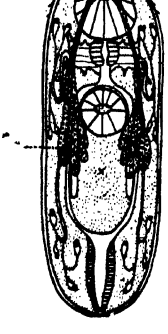

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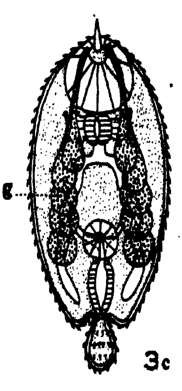

3c

Plate I. Typical stages in the development of Turbellaria and monogenetic and digenetic Trematodes, illustrating the kinds of modification effected with the assumption of a parasitic existence. 1a, young pilidium larva of a turbellarian (considerably modified from Lang); 1b, miracidium of bloodfluke, showing original adaptation to a free-swimming stage, with salivary glands for destruction of host tissue; 1c, sporocyst or metamorphosed miracidium (second generation) highly modified to meet the parasite life; 2a, Müller's larva, an indirect turbellarian type; 2b, larva of the ectoparasitic trematode, *Polystomum* (after Zeller); 2c, larva of *Aspidogaster conchicola* immediately after hatching; 2d, redia, or semi-modified parthenita of second generation; 3a, young planarian, having direct development; 3b, *Cercariacum* or tailless Cercaria (modified from Cort); 3c, microcercous cercaria of *Paragonimus westermanii.* (All drawings are original if not otherwise stated.)

(3) The *cercaria*, or tailed larva, which lives a free existence during transfer in those species having an active migration from the mollusc to the vertebrate host (Plate I, fig. 3c).

(4) The *adolescaria* (terminology of Ssinitzin, 1911),[3] or agamostome stage, intermediate in maturity between the cercaria and the adult form in the vertebrate host.

(5) The *marita* (terminology of Ssinitzin, 1911) or sexually mature flukes in the vertebrate host. They are usually referred to as the "hermaphroditic generation," but this term is not sufficiently comprehensive, for it does not include such groups as the blood flukes in which the sexes are separate.

In general, it may be stated that there are examples of all these stages of development within the mollusc, but that functional parthenitae are not known for vertebrate hosts except in the case of certain monostomes, *Cyclocoelum mutabile* (Zeder) and *Typhlocoelum flavum* (Mehlis) in which each miracidium, which hatches from the egg while still in the uterus of the marita, contains a single well-formed redia or second generation parthenita. But here it is necessary for the miracidium to reach a molluscan host before further development can take place. A much more illuminating condition obtains in the case of *Cercaria indica XV*, a fork-tailed cercaria belonging to the non-mammalian group of blood-flukes, which has recently been described by Sewell (1922). In this species the miracidium metamorphoses into a parent sporocyst (first generation parthenita) which, in turn, produces daughter generation sporocysts parthenogenetically. These daughter sporocysts have the power of producing parthenogenetically both cercaria and miracidia (*l. c.*, pl. XXXII, figs. 1, 2). While the cercaria are able to seek a vertebrate host, the parthenicolous miracidia may reinfect the molluscan host already parasitized or may swim out of the mollusc and attack and infect other susceptible molluscs. Thus we have here a clue as to the complete life cycle involving both molluscan and vertebrate hosts, and, in addition, the remarkable condition in which the abbreviated life cycle may be completed without the interpolation of the maritic generation.

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[3] Dollfus's term "metacercaria," designating this stage, is neither based on sound morphological nor on genetic evidence.

We may describe the life history of a typical digenetic trematode in some such terms as these. The miracidium, which is furnished with a ciliated epithelium for a free-swimming existence, and which, likewise, has secretory glands for digestion of host tissue, by a specific tactic stimulus, possibly that of the mucus secreted by the mollusc, is attracted to a certain mollusc in its immediate neighborhood, drives headlong toward the mollusc and at the first point of contact pours forth histolytic ferments. If the enzyme is specific and this point of contact consists of soft tissue, then an entrance into the mollusc is effected. Gradually working its way inward, the parasite reaches the lymph spaces of the host and finally arrives at the interhepatic lymph sinuses.

Primitively, the larva escaping from the egg, as in the ectoparasitic trematodes, developed *in situ* into a hermaphrodic adult worm. But under the change of conditions as an endoparasite the genital complex has become simplified and parthenogenesis has been substituted, with the production of one or more daughter individuals. Usually, the miracidium (Plate I, fig. 1b) metamorphoses into an extremely simple organism, the sporocyst (Plate I, fig. 1c), in which only ectodermal and mesodermal layers are present and in which the endoderm has disappeared. There is a body cavity which serves as a brood pouch for the developing daughter embryos. These daughter individuals then grow to maturity, escape from the mother sporocyst, and, in turn, produce parthenogenetic young. But the second generation parthenitae consist also of simple organisms, either second generation sporocysts, as in the schistosomes or xiphidiocercariae, or of rediae, which are homologous almost part for part to the larvae of the aspidobothrids, with a definite axial gradient, and with well-developed pharynx and rhabdocoel gut. (Compare figures 2c and 2d). There follows a granddaughter generation,[4] which may be another generation of rediae (described for *Fasciola hepatica*) or even miracidia (in Sewell's blood-fluke), but which are most usually tailed larvae, the cercariae. Primitively, these larvae did not exist. Even where the vertebrate become involved as a host, no doubt the transfer was made passively, the flukes being taken over into the vertebrate along with the mollusc which served as food for the vertebrate. This situation prevails to-day in the case of *Aspidogaster conchicola,* in which the maritic adult has been found in the digestive tract of fishes (*Leuciscus aethiops*) and turtles (*Amyda sinensis*), as well as gasteropods and bivalves, in which it more usually develops. But a condition must have arisen in which there came to exist on the part of the fluke a "desire for more worlds to conquer," and to meet this need a larva capable for a brief period of a free-swimming existence was produced. Unlike the miracidium, this larva possessed no cilia, so that the locomotor function was effected by the development of a caudal appendage. Certain tailless larvae of the distome group, called *Cercariaea* (Plate I, fig. 3b), belonging to the same generation as cercariae, illustrate this more primitive condition.[5] As in all other groups of animals the tail in some cercariae has become elaborated far beyond its functional justification and actually constitutes a burden to the larva. In other cases it has become modified to serve other functions.

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[4] Bucephalus and Leucochloridium apparently omit the second generation of parthenitae and produce Cercariaea within branched first generation sporocysts.

The cercaria on reaching a suitable vertebrate host has one of two possible procedures. It may penetrate the tissues of the host or it may be taken in with the food of the host. To effect the former method of invasion salivary glands have been developed in the cercaria as in the miracidium (compare figs. 3b and 3c with 1b). The human blood flukes represent a group which still use the skin method of entrance effectively. But apparently most of the trematodes were not entirely successful with this method and there remained for them the alternative of inclusion in food material. Since the life of the cercaria as a free-swimming organism is ephemeral (it usually can not digest food), and since the cercaria can not pass the acid medium of the stomach of the vertebrate without being digested, it became necessary for a second type of glandular structure to become elaborated by the larva, namely, the cystogenous glands. These glands secrete an impermeable substance around the cercaria which effectively protects it against the unfavorable conditions of the environment, whether these be desiccation, hyperacidity or merely a long period of passive existence before the larva reaches its next host. In some cases, as in *Fasciola hepatica, Fasciolopsis buski,* many monostome, amphistome and echinostome larvae, encystment occurs on vegetable matter, which offers a satisfactory means of transfer to the digestive tract of the vertebrate. Such a parasite is able to accommodate itself to a considerable number of such hosts. Another group of cercariae is able to effect penetration of the epithelial and subdermal tissues of a vertebrate or arthropod previous to encystment. This host, in turn, becomes the food of another host (in every instance known, as amniote) and serves for the fluke's transfer to a place where it may mature. Thus, in the case of *Clonorchis, Loxotrema, Opisthorchis* and certain echinostomes and holostomes, fish serve as passive carriers of the adolescent fluke, while consumption of the fish on the part of birds or mammals, as the case may be, transfers the infection to the definitive host. In the case of *Paragonimus,* fresh-water crabs and crayfish replace the piscian host during the adolescent stage. Finally, there is a third type of encystment which takes place while the cercaria is still in the tissues of the mollusc, or even in the brood cavity of the parthenita itself. This last type is highly suggestive of the mollusc being the food of the next potential host.

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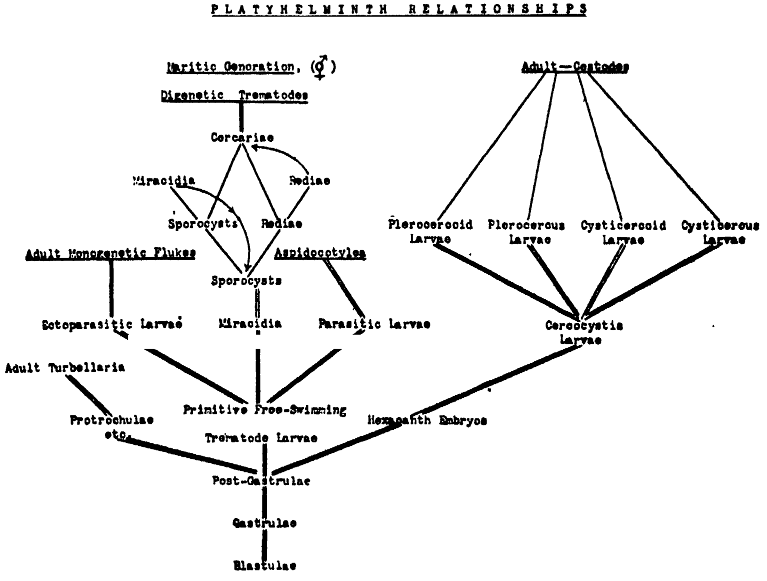
[5] Ssinitzin (1911) claims that even the Cercariaeum has the rudiment of a tail in its embryonic stage.

The adaptation of the cercaria or encysted adolescaria to the definitive host is more or less a fortuitous one. In the case of the cercaria of *Schistosoma japonicum* or *S. haematobium* there is probably no active selection of the mammalian host. The cercaria is present in the water which comes in contact with the unprotected body of the mammal. When the mammal leaves the "infected water" and the surface layer of water begins to evaporate the cercariae attempt to bore into the tissues to keep from drying up. This is not peculiarly a reaction of larval trematodes but of certain nematodes (*Strongyloides*, hookworm, etc.) as well. If the effort is successful and penetration is effected, as in the case of mammals, further development is possible. *Clonorchis sinensis* has a wide range both of piscian and of mammalian hosts. *Fasciola hepatica* may become a parasite of any herbivorous mammal. Thus one finds that specificity of hosts in the digenetic trematodes has very little to recommend it as a theory, since the host secured is more or less dependent on factors both external and internal which control the situation for the parasite.

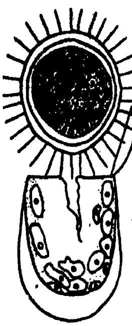


Text Figure A. Scheme of phylogenetic relationship of the parasitic flatworms.

The Cestodes

When one thinks of the cestodes or tapeworms he usually has in mind the multi-segmented ribbon-shaped worms which are commonly parasitic in the digestive tract of vertebrates. Such a form is commonly divided into "head," "neck" and "body." The "head" serves as a means of attachment, the "neck" constitutes the subapical undifferentiated region or potential growth area of the worm, and the "body" consists of immature, mature and gravid segments. The last mentioned parts are those found farthest distad.

From the point of view of parasitism as a whole and particularly that of the cestode group, this type of tapeworm must be regarded as a very highly specialized form, far removed from the more simple cestodes most nearly related to their free-living ancestors. With the cestodes, as with the trematodes, the free-living ancestor of the group is regarded as an offshoot of some form which was also the precursor of the Turbellaria. (See text figure A). This ancestor had a direct development, without metagenesis, and possibly also without even a larval stage in the strict sense of that term. Such a primitive condition exists to-day in the genera *Archigetes* (Plate II, fig. 4b) and *Caryophyllaeus,* where the development requires no cysticercus stage. That such an ancestral form was ciliated is fairly well proved by the ciliated ectoderm of several groups of cestodes of the present day. The primitive cestode may or may not have had a digestive tract. There is no evidence of such a system in cestodes to-day. Furthermore, it seems likely that if such a system ever existed in the group it was lost at an early period, since there is no evidence in the embryology of the group to warrant the belief that any of the primitive endoderm became differentiated into a digestive tube. Gastrulation is believed to occur by epibolic circumcrescence at the animal pole of the egg, a ciliated or cuticularized ectodermal layer is developed, and the remaining endodermal cells remain as undiffer-



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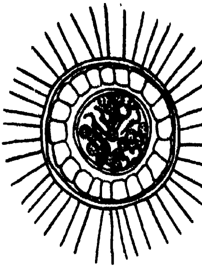

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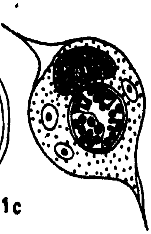

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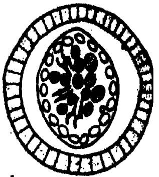

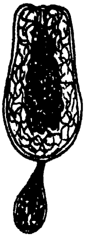

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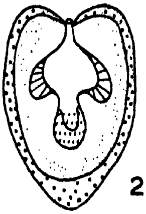

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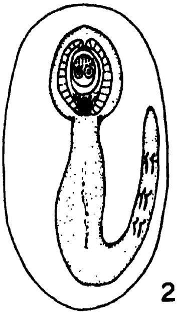

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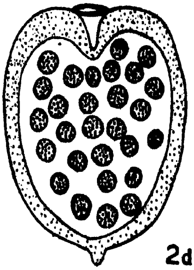

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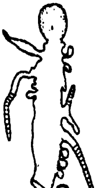

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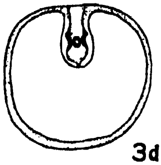

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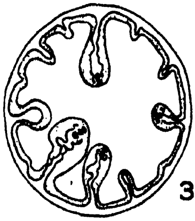

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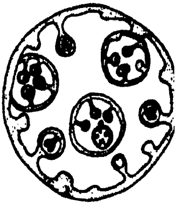

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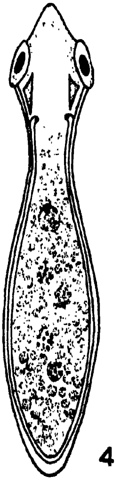

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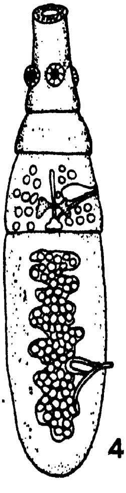

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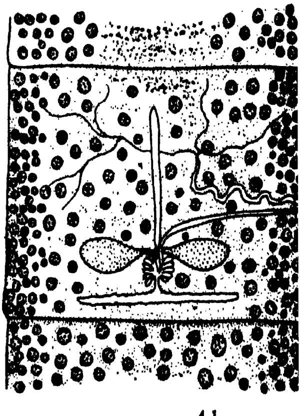

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Plate II. Typical stage showing the adaptation of cestodes to a parasitic existence. 1a, 1b, ciliated hexacanth embryos of *Diphyllobothrium latum* (after Rosen); 1c, hexacanth embryos of *Hymenolepis nana*, with polar filaments (after Stiles); 1d, 1e, hexacanth embryos of *Taenia saginata* (1d, immature, after van Beneden); 2a, procercoid of *Diphyllobothrium latum*, showing salivary glands (after Rosen); 2b, cercocystis (plerocercus) of *Dipylidium caninum* (modified from Leuckart); 2c, cercocystis (cysticercoid) of *Hymenolepis nana* (after Grassi and Rovelli); 2d, young cercocystis of *Taenia saginata* (after Leuckart); 3a, *Polycercus*, a primitive larva with exogenous budding (after Haswell and Hill); 3b, *Sparganum* (plerocercoid) of *Diphyllobothrium mansoni;* 3c, *Urocystidium gemmiparum*, showing exogenous budding and segmentation (after Beddard); 3d, cysticercus of *Taenia saginata;* 3e, coenurus of *Multiceps multiceps;* 3f, echinococcus or multiple hydatid; 4a, *Ilisha parthenogenetica*, a scolex larva (after Southwell); 4b, *Archigetes sieboldi*, a primitive cestodarian (after Mrazek); 4c, *Taenia echinococcus*, producing only one gravid and one mature proglottid at one time (after Leuckart); 4d, mature segment of *Taenia saginata*. (All drawings are original unless otherwise stated.)

entiated mesenchyme, except for the six cells which produce the hooks of the typical hexacanth embryo.

For the primitive parasitic cestodes only one host was required, probably an invertebrate. The egg was swallowed by the host, passed into the digestive tract, penetrated the wall of the mesenteron into the body cavity and there grew to maturity. However, some of these invertebrate hosts were food for vertebrates, so that before long two hosts, larval and definitive, were provided for the parasite. When such an alternation of hosts became obligatory, complications arose and the chances were very considerably lessened of the parasite completing its life cycle. To minimize this danger asexual multiplication was resorted to at two places in the life cycle, in the larva and in the adult. In the larval stage of the taenoid cestode, *Coenurus cerebralis* (Plate II, fig. 3e), in the brain of the sheep, a number of heads were budded off from the inside of the cysticercus. In the hydatid of hogs, sheep and man (Plate II, fig. 3f), not only daughter but also granddaughter heads are developed. Southwell and Prashad (1918) claim that *Ilishia parthenogenetica* (Plate II, fig. 4a) has met this difficulty by becoming a parthenita, but Woodland (1923) disputes this assumption. Recently, Professor O. Fuhrmann has shown me some of his unpublished researches in which he has demonstrated that the sparganum stage of the broad fish tapeworm, *Diphyllobothrium latum,* also multiplies asexually within the musculature of the second intermediate host. Both budding and segmentation are described for Urocystidium (Plate II, fig. 3c). On the other hand, the adult cestode has become differentiated into an elongate ribbonlike structure, divided into segments, each one of which is sexually complete, and with a subapical region (the "neck") which possesses an almost unlimited capacity for further proliferation of such segments.[6] Thus the number of segments proliferated is sometimes very great, while the egg-production is enormous, so that the chances of miscarriage between hosts are more than compensated for by the overproduction of viable embryos.

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[6] *Taenia echinococcus* (Plate II, fig 4c) represents a condition in which only one gravid segment is produced at one time.

In some cases among the higher taenoid cestodes there are two mammalian hosts, as for example, the dog and the pig, the dog and the sheep, the dog and the rabbit, the pig and man and the ox and man. In the case of *Taenia saginata* and *T. solium,* specificity of both larval and adult hosts obtains, although there are numerous cases of both in which the larval stage, commonly found in the non-human host, occurs in a similar location of man. In this connection the relation of man to Manson's Oriental tapeworm, *Diphyllobothrium mansoni,* is of unique interest. The reservoir hosts of this cestode are dogs, cats and their wild relatives. The cercocystis larva develops in *Cyclops leuckarti,* which arthropods are swallowed by frogs and snakes along with their food. There in the musculature the larva develops into the sparganum stage (Plate II, fig. 3b) and in due season is taken into the digestive tract of the definitive host. But man may become the host of either the sparganum stage or of the adult cestode. In the former instance the infected *Cyclops* is taken in with drinking water and sparganosis in man results, in which the muscles of the thigh and back become infected. In the latter instance man eats the infected frog or reptile and comes to harbor the adult worm in his intestinal tract.

Certain other cestodes of man and higher mammals require an arthropod invertebrate host. In *Dipylidium caninum* of the dog, cat and man, and *Hymenolepis diminuta* of the rat and man, a flea is utilized. But in *Hymenolepis nana* the cercocystis stage is believed to develop within the tissues of the digestive tract of the mammalian host, a condition indicative of a recent telescoping of the life-cycle.

Although the cestode is believed to have a common ancestry with the turbellarians and trematodes, it has developed along lines much more divergent from those groups than they are from each other. Multiplication in the larval stage of the cestode is asexual; parthenogenesis obtains at no place in the life-cycle with the possible exception of the one species cited above. In the adult stage asexual multiplication is pyramided upon sexual multiplication. However, the end result in both the trematode and cestode groups is the same, namely, the multiplication of individuals along with the lessening of chances of completing the life-cycle due to the alternation of two or more hosts.

THE NEMATHELMINTHES

(Text figure B)

Any attempt to treat the nematodes and their relatives in a comprehensive way is met by almost insuperable obstacles. The group is extremely large, comparable to the insects in numbers of species. Specificity of habitat or host is highly accentuated. Judging from the number of genera and species now known and the ease with which new forms can be discovered, it seems likely that only a small fraction of the existing species have come under observation. The investigator of this phylum may be compared to a speaker on the platform of a tremendous amphitheater, filled to capacity, standing so that when he looks in any one direction he can view only a very small part of his audience.

When one considers the nemathelminthes from the standpoint of the parasitologist he is confronted with additional difficulties. One may discuss "parasitic roundworms" or "free-living roundworms," but neither subject is complete without the other. To be sure, there are genera, such as Ancylostoma and Necator, which are obligatory parasites during a part of their existence, or even groups, such as the Acanthocephala, all members of which are parasites, but the majority of parasitic species have relatives living a free existence in the water or in the soil.

Viewed as a group, the parasitic roundworms are found to be much less modified because of their parasitic existence than either the trematodes or the cestodes.[7] Their adaptation has been much more easily accomplished and their life cycles much less complicated by alternation of generations or of hosts. Very few forms in the group possess a multiplicative period outside of the definitive host, and these are free-living generations of the life-cycle. If we are to believe the evidence presented by *Strongyloides,* it seems that in the free-living generation a facultative parthenogenesis exists. Both parthenogenesis and hermaphroditism are known for free-living species. However, the great majority of species depend exclusively on egg production in the adult parasitic generation for propagation. Certain parasitic nematodes are viviparous, as, for example, *Trichinella spiralis,* but similar instances obtain among free-living forms. The most interesting adaptation of the nematode ovum to a parasitic habitat is that of some of the *Filaroidea,* which live in the subcutaneous tissues, lymph spaces, or circulatory system of mammals. In *Filaria bancrofti,* which lives in lymph sinuses, the egg, usually passed into the circulatory system, is an elongate thread-like object spoken of as the filariform larva or microfilaria. In reality it is an elongate ovum, the shell of which is represented by the basal membrane covering the larva. Such larvae can pass the lymph capillaries and enter the circulatory system without difficulty, since the diameter of the larva is sufficiently small. But if the female *Filaria bancrofti* is traumatized or otherwise induced to lay immature eggs, these eggs being oval in shape can not pass the lymph capillaries, and, in part at least, are responsible for the lymph stasis which gives rise to lymphedema and elephantitis. A less advanced modification is found in *Dirofilaria immitis,* which lives in the right heart of the dog. In this species the eggs are usually laid in the immature mature (oval) stage, but develop to filariform larvae in the venous blood and later pass to the peripheral circulation. In both cases it is necessary for the larvae to reach the peripheral circulation in order that they may be dispersed. *Dracunculus medinensis,* the guinea worm of Africa and parts of Asia, utilizes a much more primitive method of dispersal. The female worm wanders into a peripheral lymph space and at a favorable time and place bores a hole through to the surface of the skin. When this infected member of the host's body, particularly the hand or the foot, is immersed in water, the female worm, which apparently has a hydrotactic reaction, utilizes this opportunity to oviposit. All these filarial nematodes require an intermediate arthropod host. In the case of the guinea worm, a *Cyclops* swallows the egg which the female worm has laid in the water, and is, in turn, swallowed in drinking water of the next host. The more highly adapted forms, however, require a transfer to be made through the bite of an insect host, in the case of *Filaria bancrofti* or *Dirofilaria immitis,* a mosquito, in the case of *Loa loa,* a mango fly. In either instance an incubation or developmental period is required in the insect, which later ejects the mature larva, which then penetrates the skin of the mammalian host. This life-history, and that of *Trichinella spiralis,* constitute the most radical modifications of the nematode parasite to its environment.

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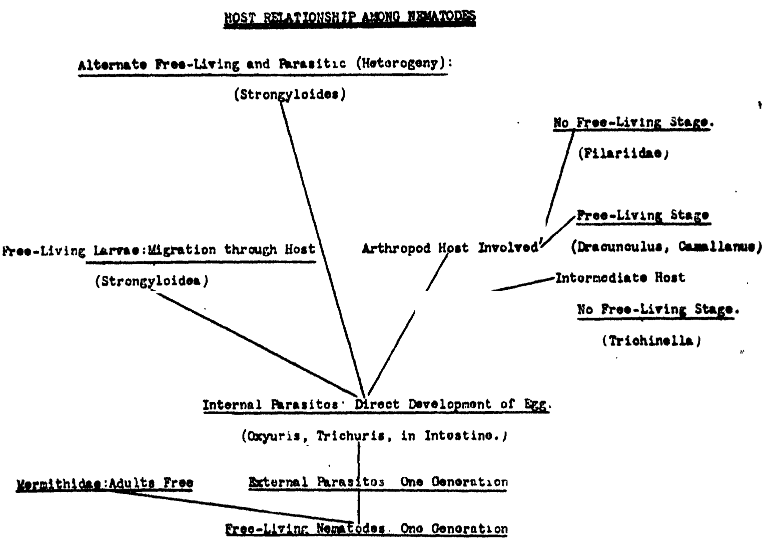
[7] In this respect they are more comparable to the parasitic protozoa than to the parasitic flatworms.

From the point of view of the entrance of the nemathelminth parasite into its definitive host, one may point out two possible routes of invasion, as in trematodes, the passive route into the digestive tract and the active route of migration through the skin. In the former instance, the eggs of the parasite may constitute a contamination of the food, which is the most usual method among the nematodes; or the larval nemathelminth, which has already become adapted to an intermediate host, may be taken into the definitive host along with the intermediate host, as is the case of all acanthocephala or thorny-headed worms in which the life-cycle is known. Some of these round worms taken into the digestive tract develop directly to adulthood, others undergo a migration through the tissues of the host before development can be completed. *Ascaris lumbricoides* is an example of the latter type. Whether this represents an old adaptation or a newly acquired accommodation is problematical. Another comparatively large group of nematodes actively enters the definitive host through the skin. As far as one is able to learn from the literature, the *Strongyloidea,* to which the hookworms, *Haemonchus contortus, Heligmosomum muris,* and related forms belong, all pass a free-living, post-embryonic existence, with one or more moults, and a change from a rhabditiform to a filariform larva (metamorphosis) and then ordinarily invade the definitive host through the skin. Likewise, the *Angiostomidae,* to which *Rhabdonema nigrovenosum* of the frog, and *Strongyloides* belong, after passing their free-living generation, all naturally invade their adopted vertebrate (definitive) host through the skin. Whether the route through the digestive tract, which small numbers of these larvae still survive, was the original method of invasion of the definitive host, still remains uncertain, but this is the most likely inference. The adaptation of the *Filaroidea,* whereby the larval stage became parasitic in Arthropoda, is a much more specialized condition of parasitism, in which both infection of the definitive host and dispersal of the worms is dependent on the arthropod. Only in the most highly specialized members of the family *Filariidae* does parasitism among the nematodes attain a degree of intimate dependence on the host which is so characteristic of the cestodes and the digenetic trematodes.

The *Mermithidae* and *Gordiacea* are unique among parasitic nemathelminths in that the larval stage only is parasitic, usually in an arthropod host, whereas the adult individuals live a free existence, respectively, in the soil or water.

The *Hirudinea,* or leeches, and species of other groups constitute cases of parasitism more or less pronounced, but in none of these groups has either the mode of life or the habitat caused such profound modifications to obtain as exist in the parasitic platodes or the more specialized filaroid nematodes.


HOST RELATIONSHIP AMONG NEMATODES
Alternate Free-Living and Parasitic (Heterogeny):
(Strongyloides)
No Free-Living Stage.
(Filariidae)
Free-Living Stage
(Dracunculus, Camallanus)
Free-Living Larvae: Migration through Host
(Strongyloidea)
Arthropod Host Involved
Intermediate Host
No Free-Living Stage.
(Trichinella)
Internal Parasites· Direct Development of Egg.
(Oxyuris, Trichuris, in Intestine.)
Mermithidae: Adults Free
External Parasites One Generation
Free-Living Nematodes. One Generation

Text Figure B. Scheme of host relationship among the nemathelminths.

GENERAL CONSIDERATIONS

When one has viewed the special groups of parasitic helminths with respect to their peculiar adaptations to the parasitic life, it is altogether fitting that certain of these phenomena be considered in the large, in order that the common trend of parasitism in metazoan invertebrates may to some extent be measured.

The subject under discussion confines one to the consideration of parasitism among helminths as viewed from the standpoint of the parasite and not of the host. As in the case among other groups of parasites an optimum condition for the parasite is one in which the number of parasites involved, their position in the host, and the secretions or excretions of the parasite do not set up a sufficiently grave pathological condition of the host to endanger its life.[8] For example, the presence of a dozen hookworms in the intestine of the host is not a matter of concern, for there are little or no clinical effects from such an infection. However, the wanderings of *Ascaris* into the liver, of *Paragonimus westermanii* or echinococcus into the ventricles of the brain, or the obstruction of an important lymph channel by a filaria may set up grave symptoms which endanger both the life of the host and of the parasite. Yet the establishment of optimum conditions for the parasite is entirely outside the powers of the parasite. Likewise, the number of individuals which completes a life cycle depends on many external factors entirely outside the control of the parasite, except as an indirect compensation is afforded by the influence of these unfavorable conditions in modifying the course of events, or the organic structure of the parasite. Thus the number of schistosome larvae entering a mammalian host is no direct index of the number which will reach the mesenteric-portal system, since fortuitous distribution through the lymphatic and circulatory systems condemns a large share of them to blind passages, there to die as foreign-body emboli within the host.

Nevertheless, it must be admitted that the majority of parasitic platyhelminths and nemathelminths have achieved unusual success in meeting the conditions of their parasitic existence. Perhaps the most extraordinary part of this whole adventure is the ability of many helminth parasites to live at one and the same time as parasites of warm-blooded vertebrates, together with a free-living existence or a parasitic life in an invertebrate host. The adaptation to differences in temperature, tonicity and chemicals in solution in the medium is even more of an achievement than the morphological changes due to differences in food supply or the elaboration of acetabula and hooks for attachment to the tissues of the host.

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[8] Some investigators regard this view as teleological. The adaptations of the helminth to the acid medium of the third and fourth stomachs of ruminants and their active resistance to host secretions may be easily demonstrated. An anesthetized but living helminth is soon digested in the same habitat in which it lives normally.

Perhaps one of the most interesting problems involved in a study of the helminth parasites is that of host selection. Primitively, many of these relationships must have been mere fortuitous contact of parasite with host, but as adaptations became more and more intimate there have come to be definite correlations in some instances and definite satisfactions in others.

As an example of the former type one needs only to cite the relationship between the trematode miracidium and the mollusc. Undoubtedly this host selection on the part of the miracidium was originally a chance undertaking. It was mere accident as to what mollusc the miracidium would attack. Many species of miracidia are known to attack molluscs without successful penetration. Certain species of molluscs are more commonly found in certain habitats than others. The constant association of miracidia of one species with certain species of molluscs in this same habitat has brought about not only a tactic response of the larvae for such hosts, but also a specificity of cytolytic substance in the secretory glands of the larva which permits it to destroy or digest away molluscan tissue, and thus gain an entrance to the lymph spaces of that host. Thus the miracidia of *Schistosoma japonicum* have become adapted to the amphibious triculinid *Rissoidae,* which enter only the surface stratum of the water, while the miracidia of *S. haematobium* and *S. mansoni* utilize an entirely different group of snails, the planorbids, which are much more cosmopolitan in their distribution and have entirely different edaphic requirements. Undoubtedly the confinement of schistosomiasis japonica to China, Japan, Formosa and portions of the Philippines is associated with the geographic distribution of the molluscan hosts utilized, else why has not the disease become established in Korea, Cochin-China, the Malay and other centers where human carriers are plentiful and where the climatic conditions are favorable, but where the particular genera of molluscs are not found?

In the second instance, the process by which cercariae of the schistosomes and the filariform larvae of the *Strongyloidea* effect penetration of the definitive host is worthy of note. In either case there are probably three factors involved, namely, moisture, temperature and chemical stimuli. In both groups, all that is necessary to effect an attack and attempted penetration of the host tissue is to place the larvae in a drop of water on the surface of the body and allow evaporation to take place. If the adaptation is specific, penetration is effected. If it is non-specific, in the case of schistosome cercariae, a dermatoid rash is at least indicative of the attempt. Some four years ago I tried out this idea by placing on my forearm a drop of water covering an area the size of a penny, containing a number of *Cercaria leptoderma,* a non-mammalian blood fluke, at times found in the edible snail in the vicinity of Peking. In the course of a half-hour the water evaporated, accompanied by a definite itching of the area and the development of a definite erythematous rash. After two hours or so the rash disappeared, while the cercariae evidently failed to secure a penetration, since no untoward consequences were later experienced. In the case of nematode larvae normally entering the host via the skin Professor F. Fülleborn has shown that they will enter any tissue in the vicinity if the free moisture of the immediate environment is withdrawn. Slight increase in temperature in all these examples, such as that effected by placing the larva on the surface of a warm-blooded animal, stimulates activity. Information on the chemotactic response of the larva for the definitive host is as yet almost entirely wanting and should be investigated. Once the larva has reached the definitive host, there is again the matter of actual penetration to be accomplished. This involves either the elaboration of an enzymatic ferment specific for digestion of the host tissues through to the peripheral circulation, or organs for mechanical abrasion and penetration, or both, and the degree of success is directly measured by the efficiency of these organs. Larvae which have not perfected these organs are unsuccessful in their efforts to effect penetration. The Oriental blood-fluke, which is potentially a parasite of all mammals exposed to the cercarial stage, is not found in the water buffalo in infected areas in China, not because the animal is immune to the disease but because the skin of the animal is too tough for penetration, whereas the native cow harbors the infection.

There is, however, a certain immunity to many helminth parasites, acquired as the host grows older. This problem has not been studied in detail, but it is known that on equal exposure children are more heavy carriers of hookworms, ascarids, whipworms, hymenolepids, etc., than are adults. Likewise, the chicken ascarid, *Ascaridia perspicillum,* is commonly "lost" by the chicken after a certain period of its life. This phenomenon is probably closely bound up with the formation of immune bodies in the host which overbalance the adaptations of the parasite for its host. In many cases there is a maximum number of helminth parasites of any one species which a host will harbor. This is apparently due to a stimulation of the host on the part of the parasite, whereby the host is able to throw off hyperinfections. That the phenomenon is due to some substance fabricated by the parasite is demonstrated by the fact that saline extracts of the parasite, when injected into the host, will frequently not only prevent additional infections but will at times cause the host to lose its original infection.

INCREASE IN EGG WEIGHT IN RELATION TO THE TIME THAT LAYING COMMENCES

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THE standard method of measuring the laying capacity of a pullet is the number of eggs laid during what is termed the pullet year of production, but it is possible that there are means of determining in advance of the conclusion of the laying year the potential producing capacity of a pullet. Hadley (1919), for instance, has developed the thesis that increase in egg weight serves as a criterion of numerical production and that it is possible to determine in advance of the conclusion of the first year of production the relative potential laying capacity among individuals in a flock of pullets.

The theme of immediate interest in this paper is a critical analysis of the results secured by Hadley in a study of egg weight in relation to production in a flock of 38 white Plymouth Rocks at the Rhode Island Experiment Station, inasmuch as the results are not in entire agreement with those secured by the writer in his study of the same problem in a flock of 60 barred Plymouth Rocks at Macdonald College. Furthermore, it is apparent, as discussed below, that in the treatment of his data, Hadley overlooked one important factor, which if taken into consideration probably would have altered materially the conclusions drawn.

The 38 white Plymouth Rocks were hatched in April, 1909, and are reported by Hadley to be of equal age and condition, presumably at the time laying commenced. He does not say when his birds commenced laying, and this is a very important point, inasmuch as it has been observed, Lippincott (1921), Atwood (1923) and Jull (1924), that there is a very significant correlation between age in days laying commences and the mean weight of the first ten eggs laid. It is unfortunate, therefore, that Hadley made no record of the month that laying commenced, and the importance of this matter will be emphasized later in dealing with the factor of increase in egg weight in relation to production.

What Hadley apparently failed to realize was that the time that laying commences is a very important factor affecting the mean weight of the first ten eggs laid. This point is clearly demonstrated by reference to Figure 1. Since Hadley has treated his matter on a monthly basis,

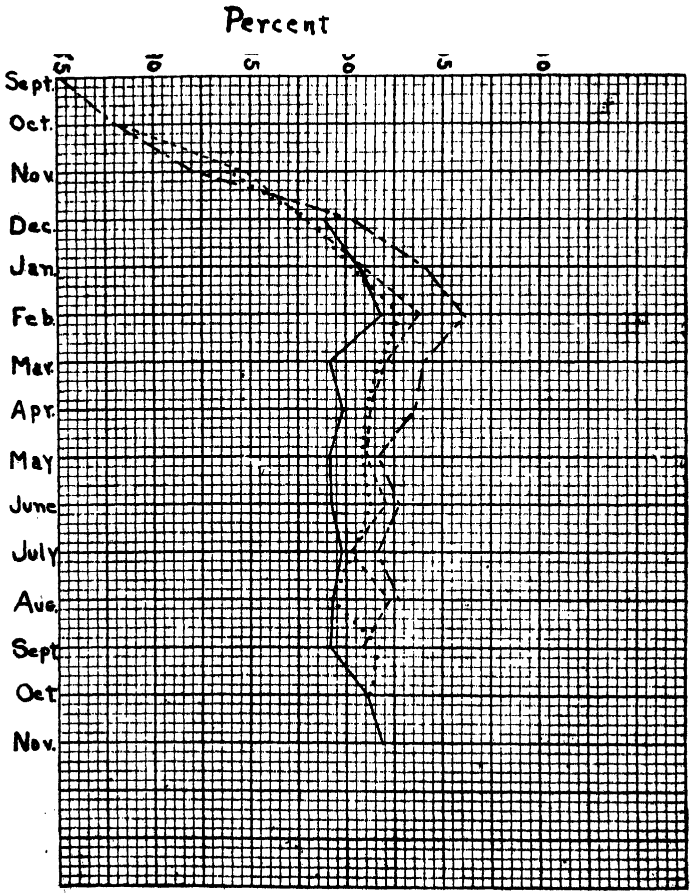

FIG. 1. Showing the monthly mean egg weights as percentages of the mean egg weights of the total production, according to the month laying commenced.

the writer has done the same, in order that the results might be comparable. It has been pointed out previously that several investigators have demonstrated the existence of a high and significant correlation between age in days laying commences and the mean weight of the first ten eggs laid. It would be expected, therefore, that the first ten eggs laid by birds commencing to lay in September would be considerably lower in mean weight than the first ten eggs laid by birds commencing to lay in December, providing all the birds were hatched about the same time.

Among the 60 birds, 17 started to lay in September, 10 in October, 16 in November and 17 in December. The eggs were weighed daily as laid, with the exception of eggs laid on Sundays, holidays and occasionally other days. The mean weight of the total annual production as well as the mean weight for each month were determined in the case of every bird. When the monthly mean egg weights for each group of birds, according to the month laying commenced, are expressed as percentages of the mean egg weights of the total production for each group and are plotted on monthly ordinates, as shown in Figure 1, the point is brought out clearly that the earlier in the season that laying commences the smaller are the first eggs laid in relation to the mean weights of the total production. From this it naturally follows that the earlier in the season that laying commences the greater would be the increase in mean egg weight of ten eggs laid in April or September over the first ten eggs laid.

In the case of the 38 white Plymouth Rocks the egg-weight curve was of a bimodal type, one mode occurring in April and one in September. In the case of the 60 barred Plymouth Rocks the egg-weight curve shows one mode only, which occurs in February. That is, in the flock of 60 birds monthly mean egg weight increased up to and including February and then it gradually decreased. It may be said that ten eggs about the middle of February were selected because February was the modal month of monthly mean egg weight, just as April and September were the modal months of monthly mean egg weight in Hadley's work.

In order to compare the April mean egg weight with the mean weight of the first ten eggs, Hadley divided his flock into two groups, one group comprising the birds which laid more than the flock mean of 120.00 eggs and another group comprising the birds which laid less than the flock mean. The first group had a mean production of 143.00 eggs and the mean percentage of increase in mean egg weight was 5.40 per cent. The second group had a mean production of 99.00 eggs and the mean percentage of increase in mean egg weight was 2.60 per cent. Not as many birds were laying in September as in April, and when the flock was divided on the same basis the group of birds, each laying more than the flock mean, had a mean production of 151.00 eggs and the mean percentage of increase in mean egg weight was 5.80 per cent., while the group of birds each of which laid less than the mean for the flock had a mean production of 105.00 eggs and the mean percentage of increase in mean egg weight was only 1.00 per cent. The writer divided his flock into two groups, the first comprising the birds which laid more than and the second comprising the birds which laid less than the flock mean of 152.40 eggs. The February mean egg weight was compared with the mean weight of the first ten eggs laid. The first group, 34 birds, had a mean egg production of 178.54 ± 2.18, and the mean percentage of increase in mean egg weight was 12.75 ± 1.06. The second group, 26 birds, had a mean egg production of 118.31 ± 3.59 and the mean percentage of increase in mean egg weight was 8.31 ± 1.66. The results obtained by the writer are in general agreement with those obtained by Hadley and seem to show that the birds with the greatest percentage of increase in mean egg weight were the birds that laid the greatest number of eggs. On the other hand, in a further examination of the results secured by the writer, it is observed that the difference in production between the first and second group is 60.23 ± 4.20, while the difference in mean percentage of increase in mean egg weight is 4.44 ± 1.96. The difference in production is highly significant, while the difference in respect to mean egg weight is not significant. Furthermore, when the month that laying commenced is taken into consideration it is found that in the first group 12 birds commenced laying in September, 6 in October, 9 in November and 7 in December, while in the second group 5 commenced laying in September, 4 in October, 7 in November and 10 in December.

Hadley next considered the relationship of the mean weight of ten eggs selected as closely as possible to the periods of absolute April and September maxima, respectively, to the mean weight of the first ten eggs laid. In both cases he divided his flock into two groups, the first group comprising those birds which showed an increase of 6 per cent. or more in the mean weight of the April and September ten eggs, respectively, over the mean weight of the first ten eggs laid, and the second group comprising those birds which showed an increase of less than 6 per cent. or a decrease. For April, there were 13 birds that showed an increase in mean egg weight of 6 per cent. or more and their mean annual production was 138.00 eggs, while there were 24 birds with an increase of less than 6 per cent., or a decrease, and their mean annual production was 114.00 eggs. For September, in the first group, there were 12 birds, with a mean annual production of 139.00 eggs, while in the second group there were 19 birds, with a mean annual production of 112.00 eggs. In order to secure results for comparison with those of Hadley, the writer determined the percentage of increase in the mean weight of ten eggs laid as nearly as possible around the middle of February over the mean weight of the first ten eggs laid. On this basis of consideration there were 38 birds that showed an increase in mean egg weight of 6 per cent. or more and their mean egg production was 155.89 ± 3.42, while there were 22 birds that showed an increase of less than 6 per cent., or a decrease, and their mean egg production was 146.45 ± 6.95. Here again the results are in general agreement with those obtained by Hadley, but the difference in mean egg production is 9.44 ± 7.74, which is not significant. Moreover, if the birds of the two groups are classified according to the month laying commenced, it is found that in the first group 17 commenced in September, 10 in October, 8 in November and 3 in December, while in the second group there were none that commenced in September, none in October, 8 in November and 14 in December. This suggests quite clearly that a division of the flock according as the mean percentage of increase in mean egg weight is more or less than 6 separates fairly completely the birds that commenced laying early from the birds that commenced laying late.

It is necessary to test further the matter of increase in mean egg weight in relation to production according to the month laying commenced, and for this purpose the results are shown in Table I. The column "Mean Percentage of Increase in Mean Egg-weight" refers to the percentage of increase in the mean weight of ten eggs laid as nearly as possible around the middle of February over the mean weight of the first ten eggs laid.

TABLE I

SHOWING THE MEAN EGG PRODUCTION AND THE MEAN PERCENTAGE INCREASE IN MEAN EGG WEIGHT ACCORDING TO THE MONTH LAYING COMMENCED

Month laying commenced	No. of birds	Mean egg production	Differences	Mean per cent. increase in mean egg weight	Differences
September	17	153.00 ± 4.59		23.00 ± 0.87	
			7.60 ± 7.32		8.28 ± 1.43
October	10	160.60 ± 5.59		14.72 ± 1.14	
			7.80 ± 9.27		7.80 ± 1.50
November	16	152.80 ± 7.40		6.92 ± 0.98	
			6.21 ± 10.57		6.30 ± 1.38
December	17	146.59 ± 7.55		0.62 ± 0.98	

From Table I it is apparent that the differences in mean egg production, according to month laying commenced, are not significant, and it may be added that the difference between the October group, showing the highest mean egg production, and the December group, showing the lowest mean egg production, is 14.01 ± 9.39, which is not significant. The fact is borne out, therefore, that in this group of birds there is no significant correlation between month laying commenced and annual production. It is apparent, however, that the differences in mean percentage of increase in mean egg weight, according to month laying commenced, are significant in every case, and it may be added that the difference between the highest, September, and the lowest, December, values is 22.38 ± 1.31, which is very significant.

This situation demonstrates that percentage of increase in mean egg weight is a far more significant criterion of the approximate time laying commences than it is of numerical production. This particular point is borne out very clearly by reference to the coefficients of correlation which have been determined. The correlation between egg production and percentage of increase in mean egg weight, 0.157 ± 0.085, is positive but is not significant, and Hadley failed to demonstrate the significance of his apparent correlation. Between month laying commenced and egg production there is a slight minus correlation, — 0.007 ± 0.087, but it is not at all significant. On the other hand, between month laying commenced and percentage of increase in mean egg weight there is a very high correlation, 0.911 ± 0.015, and it is very significant. There can be no question, therefore, that time laying commences is a very important factor affecting increase in mean egg weight and it is very unfortunate that Hadley did not take the question of time laying commenced into consideration.

In conclusion, it may be said that increase in mean egg weight can be considered a criterion of numerical production only in so far as time laying commences is a criterion of numerical production. Furthermore, it is highly probable that as a possible criterion of numerical production increase in egg weight is of an incidental character.

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A COMPARATIVE RACIAL STUDY OF THE STRUCTURAL ELEMENTS OF HUMAN HEAD-HAIR

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FROM recent studies of the structural units of the hair of mammals below the *Hominidae*[1] it has been shown that these units are characteristic rather of the diameters of the hair shafts in which they occur than of the species of mammals bearing the hair. With respect to the structural parts of the outermost integument of the hair-shaft, *i.e.*, the cuticular scales (Fig. 1), it was found that the scale-forms, as expressed by the *scale-index* (a mathematical expression of the relationship between the proximo-distal diameter of the free surface of the scale and the diameter of the hair-shaft (Fig. 3)) bore relation, not to the species, nor to the group to which it belonged, but to the size of the hair. Without using the terms in their strict mathematical sense we may say that the width of the cuticular scales and the diameter of the hair shafts vary inversely. Or, roughly, the coarser the hair, the finer the scales; and *vice versa.*

It was further found that the forms assumed by the medulla, the central column of cells of the hair shaft (Fig. 5) likewise vary with the diameter of the hair shaft, in a similar way.

Scale and medulla form are thus shown to be intimately related to the diameter of the hair shaft, in a general way. Specific and group characteristics of the minuter configurations in these two structural elements exist, however; characteristics of sufficient magnitude and definiteness to be useful to the trichologist.[2]

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[1] Hausman, L. A., "Further studies of the relationship of the structural characters of mammalian hair," AM. NAT., Vol. 58, Nov.–Dec., 1924, p. 544.

[2] *Ibid.*, "Structural characteristics of the hair of mammals," AM. NAT., Vol. 44, Nov.–Dec., 1920, p. 496.

The cortex of mammalian hair seems to be quite uniform throughout the class and consists of elongated, shrunken cells, whose keratized cytoplasm frequently exhibits traces of nuclei. This whole element of hair-shaft structure is usually an almost homogeneous and hyaline mass.

The pigment granules, both of the cortex and the medulla, are deserving of much study. In the *infrahominid* mammals many specific differences with respect to their color value, hue, size and placentation obtain.[3] Studies of the pigment granules in human head-hair are being carried on in the writer's laboratory.

The hitherto unsuspected relationships between the hair-shaft diameter and its structural units, which the studies just mentioned have revealed, suggested that similar investigations be undertaken of the status of these same elements in the head-hair of the various races of mankind. Accordingly, a collection of scalp-hairs representative of all the existing races of man was made from various sources[4] and a comparative study of the struc-

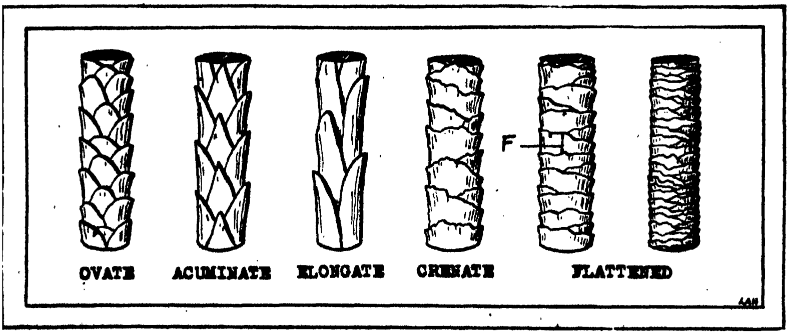

FIG. 1. The five types of imbricate cuticular scales of mammalian hair. (The coronal scales, not shown, form a distinct group.)[6] The figure on the extreme right is of a portion of a shaft of a typical human head-hair. At F is shown the method of measuring the proximo-distal diameter of the free surface (*i.e.*, the width) of the scales.

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[3] *Ibid.*, "Hair coloration in animals," *Sci. Mon.*, Vol. 12, March, 1921, p. 215.

[4] The writer is greatly indebted to the following persons for the samples of head-hair they have sent him, and heartily thanks them: First, and especially, Dr. Aleš Hrdlička, of our National Museum in Washington; Dr. Chi

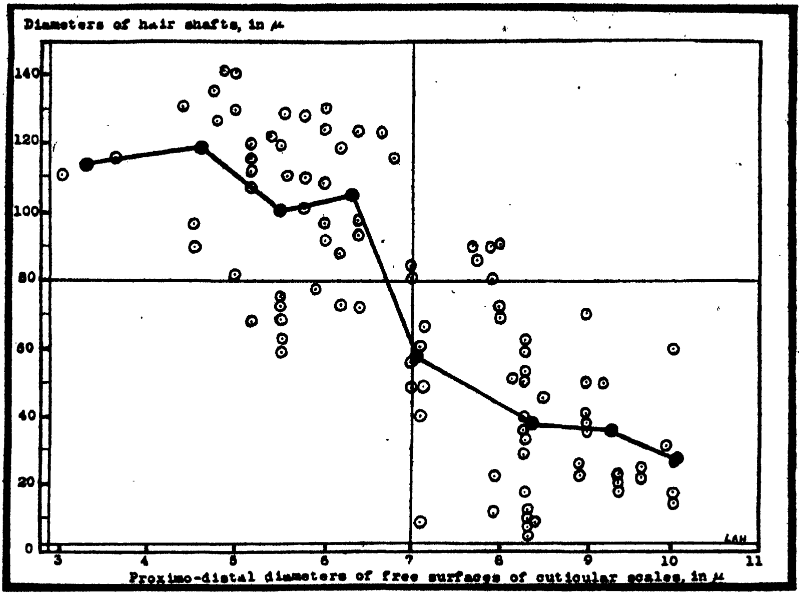

FIG. 2. Relationships between the sizes (*i.e.*, widths, or proximo-distal diameters of the free surfaces) of the cuticular scales and the diameters of the hair-shafts, as shown in 94 specimens of human head-hair from individuals representing all the existing races of mankind.[7] Clear circles represent the individual data. The determinations were made, in each case, midway between the base and the tip of the hair-shaft.

tural elements made. The results of the studies of the cuticular scales and medullas are here presented.

The earliest comprehensive microscopic study of human hair was made by Pruner-Bey in 1863 and 1864.[5]

Ping, of the National Southeastern Teachers' College at Nanking; Dr. Arnold E. Lundie, of Idutywa, Cape Province, South Africa; Professor E. W. Stafford, of the Mississippi College; The Metropolitan Museum of Art, for a splendid series of mummy hairs from Egypt; Dr. T. C. Nelson, of Rutgers University; Dr. H. D. Reed, of Cornell University; Dr. A. L. Kroeber, of the University of California; and Dr. F. G. Speck, of the University of Pennsylvania, and many others.

[5] Pruner-Bey, "De la chevelure comme characteristique des races humaines, d'après des recherches microscopiques," Memoires de la Société d'Anthropologie de Paris, Vol. 2, 1863, p. 1; and Deuxième Serie d'Observations sur la Chevelure, *ibid.*, Vol. 3, 1864.

A general micrological survey of the hairs of men and beasts was made in 1884 by W. Waldeyer, in his "Atlas der Menschlichen und Tierischen Haare, etc." (Lahr). A copy of this work was kindly presented to the writer by Mrs. John A. Roebling, of Bernardsville, N. J.

He is chiefly to be remembered by his discovery of the relation between the trans-section of the hair and its degree of curliness. He recorded the fact that the more curly the hair, the more elliptical was its cross-section; and the more nearly straight the hair, the more nearly circular its cross-section. He noted that the thinnest and flattest hairs are those of the Bosjemans, Papuans and Negroes; and the most cylindrical those of the Polynesians, Malays, Thibetans, Siamese, Japanese and American Indians and Eskimos. Pruner-Bey also recorded his observations on the variability of the medulla-forms in various hairs, but was unaware of the relationships of these forms to the shafts in which they occurred. Supposing these differences in form to be constant, with relation to groups of peoples, he proposed that hairs should be classified on the basis of the medulla-status, as follows: (1) Hairs in which the medullary canal is empty of all substance, (2) hairs in which the canal is filled with substance and frequently terminated in the shaft, and (3) hairs in which the canal is absent altogether. The first form is the present writer's *continuous medulla,* and the second form, the *fragmentary, fractional* and *broken medullas* (Fig. 4).

The cuticular scales of human head-hair are all of the flattened type,[6] and like the scales of the *infra-hominid* hairs (Fig. 1) vary, in a rough way, inversely with the diameter of the hair shaft. Fig. 2 shows graphically the relationships between the width (*i.e.,* the proximo-distal diameter of the free surface) of the cuticular scales and the diameter of the hair-shafts of ninety-four specimens of hair, representing the existing races of mankind.[7]

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[6] A classification of the cuticular scales of mammalian hair will be found in Hausman, L. A., "Structural characteristics; etc.," footnote 2.

[7] The following ethnographic classification has been used by the writer, and is to be regarded by the reader, not otherwise than as merely a convenient check-list:

I. European Race.
  (1) South Mediterranean Branch
  (2) North " "

II. African Race
  (1) Negrillo Branch
  (2) Negro "
  (3) Negroid "

This is significant, for it means that the scale-forms are, therefore, unrelated to race. For it was found that the scales varied on contemporaneous hairs from the same head, and on hairs from the same head at different periods of life, when the diameters of these hairs varied. Moreover, differences exist along the same hair, from base to tip. This is interesting, in view of the fact that many *infra-hominid* hairs show this characteristic very much more markedly. An extreme instance of this change in character from the base to the tip of the hair, involving not merely the cuticular scales but the other elements of the shaft-structure as well, is furnished by the protective or over-hair of the Platypus (*Ornithorhynchus anatinus*).[8] Such changes are explainable from the behavior of the epidermal cells at the base of the hair follicle. When a hair is about to be shed,[9] the papilla atrophies, and the bulb of the hair becomes cornified and hard. Growth ceases, and the hair-shaft is carried outward toward the mouth of the follicle by the continued proliferation of the cells of the root-sheath. A new papilla now develops, and soon becomes surrounded by the cells forming the bulb of the new hair. The early proliferation of the cells forming the tip of the new hair produces a shaft of less diameter than that which succeeds, and which is the product of the increasing mitotic activity of the hair cells. When the papilla begins to atrophy, the shaft of the hair becomes smaller. Hence hairs very frequently show a narrow or even pointed tip, a broader middle portion, and a narrowing base. The

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III. Asiatic, or Mongolian, Race
    (1) Sinitic Branch
    (2) Sibiric "
IV. American Race
    (1) Northern Branch
    (2) Central "
    (3) Southern "
V. Oceanic Race
    (1) Negritic Branch
    (2) Malayic "
    (3) Australic "

[8] Hausman, L. A., "A micrological investigation of the hair structure of the *Monotremata*," *Am. Jour. Anat.*, Vol. 27, No. 4, Sept., 1920, p. 463.

[9] Unna, P. G., "Beiträge zur Histologie u. Entwicklungsgeschichte der Menschlichen Oberhaut," etc., *Arch. f. Mikro. Anat.*, Vol. 12, 1876, p. 665.

average life of a continuously growing hair in the human scalp (that is, the period during which there is uninterrupted mitosis of the hair cells at the base of the follicle) is said to be between five and six months.[10]

Fig. 3 shows the relationships between the scales and the hair-shaft diameters as revealed by an examination of 185 species of *infra-hominid* hairs.

The human head-hairs show but one type of scale, as has been said, the flattened type. This is interpreted as being the cuticular scale indication that mankind is a single species. No differences in this type of scale, analogous to the specific differences to be found in the *infra-hominid* hairs, were encountered.

The medullas of human head-hairs varied also, but, like cuticular scales, not with race, but with diameter of hair-shaft. Fig. 4 records the results of the study of 122 specimens of head-hair, representing the five races of our table.[7] The greater number of the finer hairs lack rather

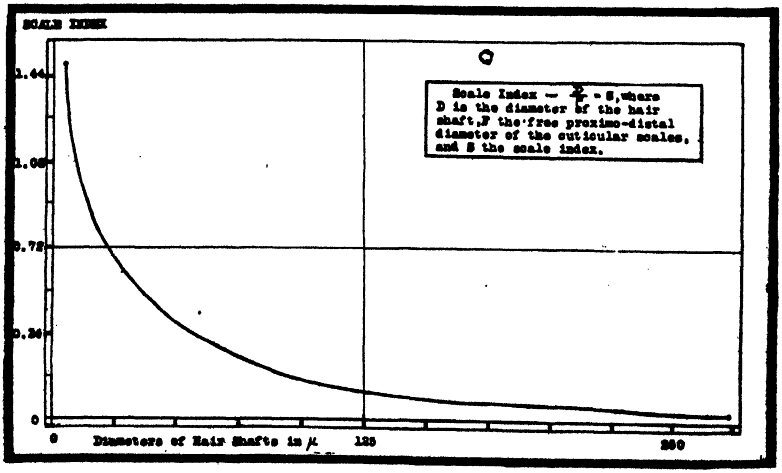

FIG. 3. Relationships between the sizes (*i.e.*, widths, or proximo-distal diameters of the free surfaces) of the cuticular scales and the diameters of the hair-shafts, as shown in 185 species of mammals below the *Hominidae* and representing all the existing orders except the *Cetacea*. The scale index, here recorded, is simply an expression of the ratio between the width of the scale and the diameter of the hair-shaft. The determinations, in each case, were made midway between the base and the tip of the hair-shaft.

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[10] Stöhr, Text-book of Histology.

than possess a medulla; and the greater number of the coarser hairs possess one rather than lack it. Intermediately sized hairs bear the various types of fragmental medullas shown in the figure. The relation between medullas and hair diameters among the *infra-hominid* mammals is shown by Fig. 5.

It is interesting and significant from the standpoint of what has been said regarding the close parallelism between both scale and medulla form and diameter of the hair-shaft to note what a comparison of these two curves reveals: (1) That in both groups the hair-shafts of about 100 microns in diameter bear virtually the same type of medulla and (2) that the *hominid* hairs do not show the *discontinuous* or *intermediate* types exhibited by the *infra-hominid* species.[11] From a series of studies of the finest hairs of very young children and of the hairs of the lanugo (hairs ranging in diameter from 10 to 30 microns) it was found that in the great majority of cases the medulla was absent. It is noteworthy, therefore, that in the case of the *infra-hominid* hairs the medullas begin to appear (in the form of the *discontinuous* medulla) when the hair shafts have attained a diameter of about 15 microns; and that in the *hominid* hairs they do not begin to appear until the hair has reached a diameter about twice as great.

The fact that the human head-hairs do not show the *discontinuous* or the *intermediate* types of medullas (those types which are so common among the *infra-hominid* mammals) and exhibit virtually no varietal differences away from the types shown in Fig. 4 is to be understood, it is believed, as being the medulla expression of the unity of the human species.

SUMMARY

The cuticular scales and medullas of the human head-hairs are related, as are the same elements in the hairs of

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[11] Many of the *Primates* exhibit the *discontinuous* and *intermediate* types of the medulla.

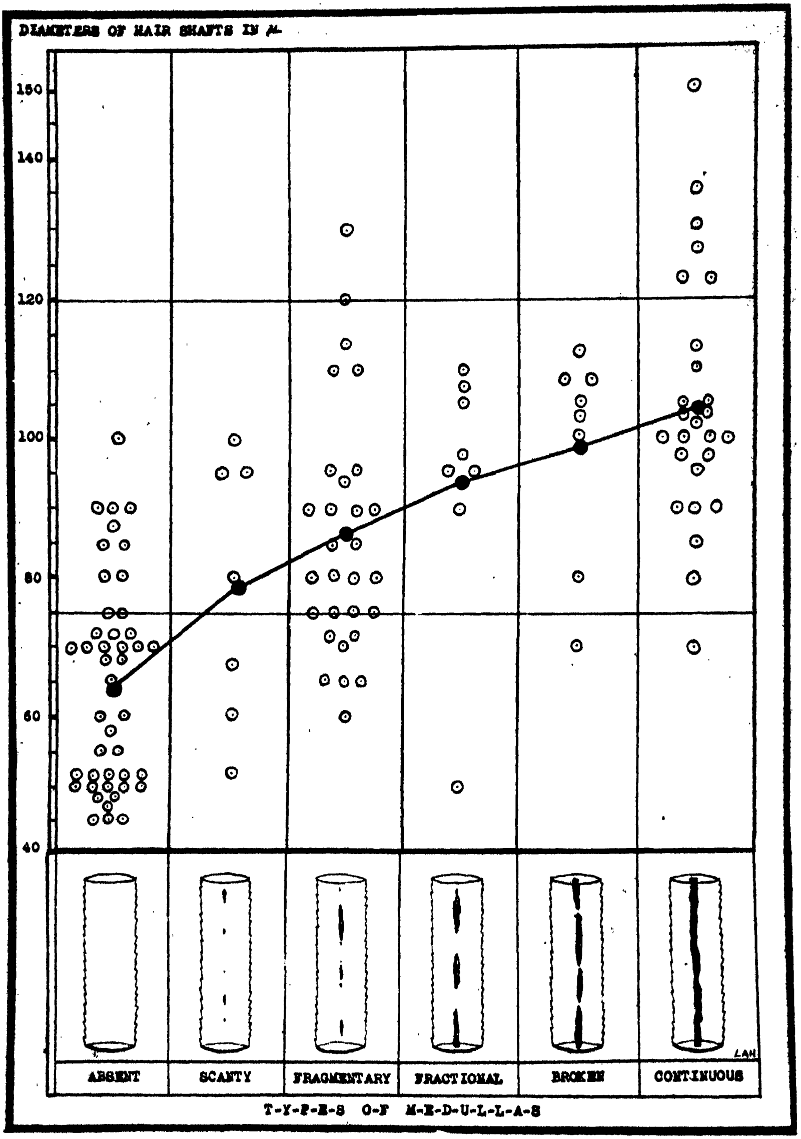

FIG. 4. Relationships between the medulla forms and the diameters of the hair-shafts as shown in 122 specimens of head-hair from individuals representing all the existing races of mankind.[7] Clear circles represent the individual data. Along the abscissa are pictorially defined the terms descriptive of the conditions of the medulla. The determinations were made, in each case, midway between the base and the tip of the hair-shaft.

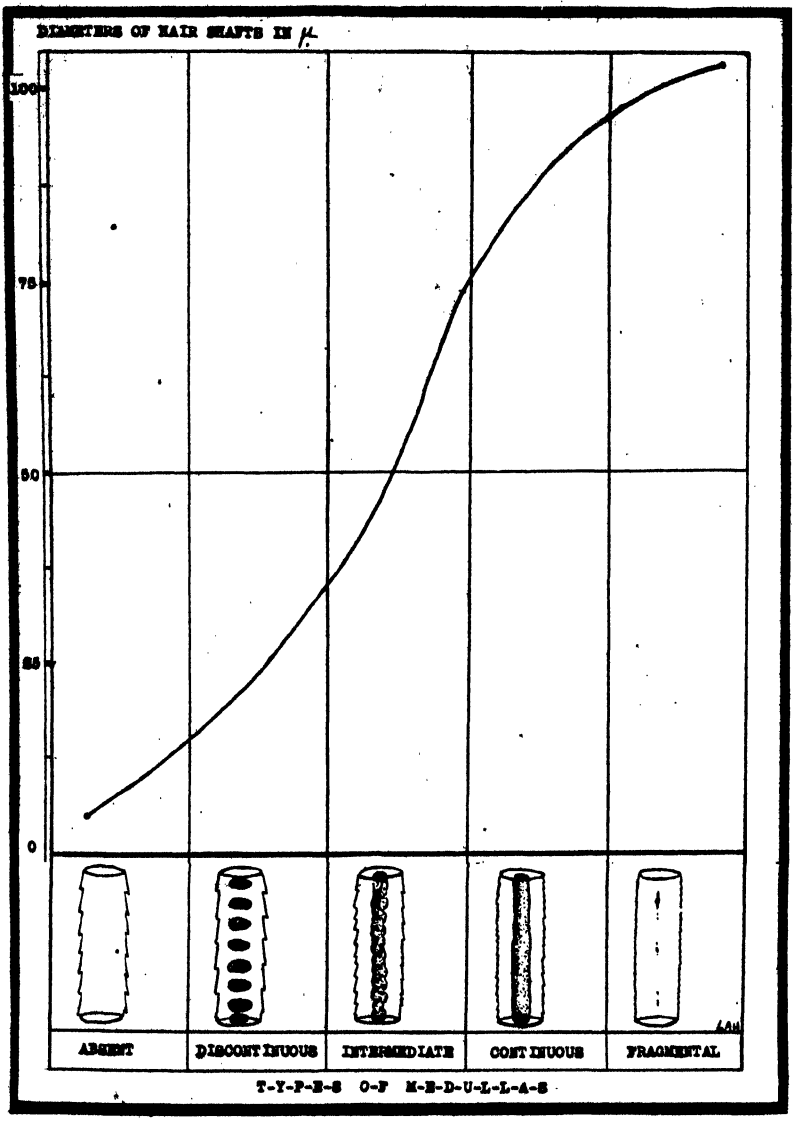

FIG. 5. Relationships between the medulla forms and the diameters of the hair-shafts as shown in 170 species of mammals below the *Hominidae,* and representing all the existing orders except the *Cetacea.* Along the abscissa are defined the terms descriptive of the conditions of the medullas. The determinations, in each case, were made midway between the base and the tips of the hair-shaft.

the *infra-hominid* mammals, to the diameters of the hair-shafts. They can not be considered, primarily, as characteristic of race.

Human hairs have not been found to show any but one type of scale, *i.e.*, the flattened, nor any but the continuous and the varieties of the fragmentary medullas.

Neither the scales nor the medullas of human head-hair show any of those characteristic modifications of form which have been interpreted as being specific, or group, differences among the hairs of the *infra-hominid* mammals.

It is believed not to be possible to identify individuals from samples of their hair, basing identification upon histological similarities in the structure of the scales and medullas, since these may differ in hairs from the same head, or in different parts of the same hair. "Accidental," or unusually striking similarities can not, of course, fall within this category.

The gross qualities of human head-hair, *en masse*, seem to be much more accurately characteristic of ethnological groups of mankind (or at least more definitely usable) than do the microscopic units of the individual hair-shafts.

THE MIGRATION OF ANIMALS[1]

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> The behavior of the very highest animals is chiefly determined by intelligence and reason. But the behavior of by far the largest number of animal kinds is mostly determined by instinct.

WITH the above quotation a well-known scientist begins his review of a recent book. The present writer begins his article with the quotation principally because he does not agree with it. To consign activities of organisms for which we have no scientific explanation to the realm of instinct, thereby removing them from the realm of the zoological to which animal activities logically belong, is both a mistake and an unscientific procedure. We know little or nothing regarding instincts; the term is often used to cover a multitude of ignorance, a wealth of known facts for which we have at present no scientific explanation. By consigning these activities to the category of instincts we save ourselves the embarrassment of admitting ignorance, but thereby remove from the field of zoological investigation many problems essentially zoological. An instinct defies scientific investigation; we can not explain it on the basis of known facts, and a problem solved by obscuring it under the blanket of instinct is thus effectually buried and is no nearer solved than before the internment, though it is safely out of the way —at least for a time! To assign a reaction to the category of instinct should be the last, final act of the zoologist, resorted to only after every line of scientific investigation has failed to throw light upon the subject, and even then it must be distinctly understood that problems so assigned are merely in a temporary dwelling. "In-

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[1] Contributions from the Zoological Laboratory of the University of Illinois, No. 258.

stinct'' solves nothing: it but adds one more problem to problems already difficult enough, though it often throws a veil of misplaced conviction of solution about a problem. The migration of animals too often is classed as instinctive. The phenomenon of migration has still not been explained in most cases, as among the birds, for example, though literally dozens of explanations have been brought forward. The present paper does not purport to offer much that is new, but is intended rather to bring before the reader for his consideration a line of thought which the writer believes is too little considered, yet a line which is open even now to scientific investigation as yet untouched, and which is, in the course of time, subject to proof or disproof.

Migration is ordinarily defined as ''a *periodic* passing from one place to another.'' At once two types of migrations present themselves: (1) daily movements and (2) seasonal movements. The difference between them is this: daily movements are primarily feeding movements, or reactions to light, while the seasonal migrations are ordinarily breeding movements. While the writer wishes to concern himself primarily with the second of these types, it is perhaps worth while to briefly enumerate one or two examples of the first type, the daily migrations, to which we have a more or less satisfactory biological answer, and which are ordinarily not classed as instinctive.

These daily migrations must be admitted truly migrations, yet they are of essentially a different type from the seasonal movements—that is, their *cause* is different. There is less mystery about them because we can make some reasonably scientific explanation for them. These daily migrations are, as has been indicated, of two types: (1) those movements which are due to light reactions on the part of the organisms and (2) those which are feeding reactions. True, this latter type may, in many cases, trace back to the former in the last analysis, yet the *causing* factor is quite different, though the *ultimate* factor may be the same. As an example of migrations of the first category, we may cite the movements of the entomostraca. It is known that many species move upward at night and downward during the daytime. This is evidently a migration and a light reaction, as has been proved. At the present time we can go little beyond saying that the movements are due to a negative phototropism on the part of the organism, and by this we mean that the organism is physiologically not attuned to light and therefore avoids it. One would hesitate long before assigning instinct as the causing factor for the movement of *Daphnia* and *Cyclops*. As an example of the second type, the feeding movements, the writer cites the case which has come under his personal investigation, that of the cisco (*Leucichthys artedi*), the small lake herring found throughout the northern Mississippi drainage area. Entomostraca form a very large percentage of the food of these fish—almost 100 per cent. during certain months —and it has been found that these fish likewise are up at night and down during the day, corresponding to the movement of their food. To this type of migration, then, we likewise have an answer.

We turn now to the seasonal migrations, which are admittedly associated in some way with the breeding reaction. Among the fishes we have many examples. The salmon migration is well known. The pickerel (*Esox lucius*), leaves the deep waters of the lake as the ice breaks up, and spawns in the very shallow water of the marsh overflow. Among the reptiles we have the sea turtles, ordinarily shunning land, coming to shore to lay the eggs, and then returning to the open sea again. Perhaps the best known seasonal migration is that of the birds, details of which need hardly be presented here. The *facts* of bird migration are well known and well established; the cause of the migrations is not so clear.

Concerning this phenomenon of migration among birds, what explanations are offered to cover the facts? There have been so many ideas advanced that only a few of the better known are offered:

(1) *Food:* That food is the underlying factor causing bird migration is still strongly held by many observers. Undoubtedly food is a factor in some cases, but not in many. The ducks and geese go south in the fall with the closing of the lakes, but why did they go north in the spring? All birds leave the south when food is abundant and most return south again while food is still abundant in the north. Were food the factor causing migration, once south the birds would never come north!

(2) *Temperature:* That temperature determines migration is also strongly held. It has been shown that the robin comes north more or less coincidently with a certain temperature wave. Yet no one has explained *why.* The determining temperature in the case of the robin is said to be 35 degrees Fahrenheit, yet robins winter where the temperature is well over this point many times during their sojourn in the south: why do they suddenly come north with it? Again, the swallows go south while it is still very warm.

(3) *Wind:* It has been shown that migrations occur during winds of certain directions. I do not question the fact: I admit it, but I ask again, *why?* Surely during their stay in the south the migrants have been subjected to winds from every direction again and again; why did they not come north with that critical wind before?

(4) Humidity, pressure, etc., and a host of other factors have been advanced to explain migration, yet the *how* and the *why* is not clear. The same may be said of the idea most recently advanced, that birds migrate in the direction of the lengthening day. Nothing is here explained: one problem is substituted for another.

(5) *Breeding instinct:* Here we come to the "zoological buck-passing." Birds come north to breed and when they are done they go back south again. Simply perfect, but not, alas, perfectly simple! This would be, according to the quotation cited, the final answer. I offer that it is unsatisfactory.

(6) Physiological changes going on within the body, mainly in the sex organs. Here is the crux of the whole

matter, and the hub around which this paper turns. We shall deal with this at some length shortly.

The point that I would make is this: that, with the possible exception of the sixth, not one of these answers explains *why* birds migrate, and the sixth is not very clear about it. I believe that they fail to explain it because not one of them takes the bird *itself* into consideration; all are attempts to explain the movements as due to external factors only. And this is but natural perhaps, because we know a good deal about externals and very little indeed about internals! Yet I believe that the internal factors are of the utmost importance. Not that I would discard the external influences, but I would erect internal factors upon which the external conditions may operate. Not food nor temperature nor wind nor length of day explain migration *unless there is an internal mechanism upon which they can operate.* Then, and only then, may one or all of them operate, not as causative agents, but as secondary effecting or contributing factors. So much for the theories of migration. Let us now turn to a consideration of other matters which will lead us back eventually to the subject in hand.

Since migration concerns the distribution of animals, we may well ask the question: What determines the distribution of animals? While there are several answers to the query, the one with which we are concerned may be discussed very briefly under the title of "regulatory mechanisms." We are not concerned with physical barriers as limiting agents.

Those who have studied along the lines of physiological ecology are familiar with the fact that the animal body is a complex of regulatory mechanisms which regulate the body activities of the animal and, through these, may regulate the distribution of the species. Such regulatory mechanisms as those associated with respiration, hydrogen-ion concentration, enzyme, water and temperature regulation are known. Only one of these will be given as an example to illustrate my point: the respiratory regulation. Let us take the case of the mountain sheep which lives in the high altitudes and which has no physical barrier to keep it from coming down into the plains. But it does not come down. When the sheep descends to a certain level it turns around and goes up again. Why? As the animal descends the mountain, the air becomes denser and, to borrow an apt expression from Dr. Shelford, "its carbureter is regulated to light, high air." As it comes down, the sheep gets too much oxygen and the respiratory rate is decreased, as animals have a capacity to use only a certain amount of oxygen. The animal simply can not live under the new conditions, and must return to conditions suitable to it as it can not adjust to the new conditions. In our own case the converse is equally true: when we go up into high altitudes we can not attune to the light oxygen pressure. At high altitudes the solids in the blood increase, due to a transfer of fluids into lymph. When the blood contains too much lactic acid this acid affects the respiratory center and causes difficulties. Were man not a reasoning animal he would be barred from high altitudes as the sheep is from the low.

These various regulatory mechanisms are all bound up together, with the result that every animal has the capacity to regulate its conditions, but this regulation is limited to the usual fluctuations of its natural and normal environment. When fluctuations pass beyond that point the animal can not adjust to them. Thus an animal regulates through behavior by the following steps: (1) Certain processes are going on within the animal; (2) external changes affect the rate of these processes and upset them; (3) the result is movement of the animal in an attempt to adjust to the changes; (4) these movements bring the animal into various conditions, one of which may relieve the disturbance, in which case the disturbing factor being removed, movement would cease, as the equilibrium between processes and environment is established. By these steps we see that the environment of an animal may be regulated by behavior, *e.g.*, movement.

These statements all assume changes in *external* conditions, which change is followed by movement of the animal due to the upset of the equilibrium that must exist between the internal processes and the external conditions. The animal is comfortable only when it is in a state of stable physiological equilibrium with its environment. External change upsets this condition. Now let us ask this question: *Suppose* the change should be *internal* instead of external: would not the same results be manifested? Such an animal is no longer in a state of congenial physiological equilibrium with its environment. The conditions are the same in both cases: the carbureter is out of adjustment to the conditions! Would not movement result as in the former case? I believe it is logical to expect that it would. Therefore, if we could establish the existence of periodic internal changes going on coincident with the periodic movements of the animal, we might demonstrate the movement as being caused by such changes. Such, at least, would be a possibility. Since migration is an example of this periodic movement, then we might explain migration as being caused by these internal changes. Are there in reality any changes going on at the time of migration? Can we establish internal changes sufficiently marked to throw the organism out of equilibrium with its environment? I believe we can, and I think that we can go farther: I think we can show what is *causing* the internal change. Since these migrations with which we are dealing are admittedly breeding or reproductive in nature, let us turn to a consideration of the reproductive cycle.

To the student of modern ecology the term "physiological life cycle" is well known. By it is meant the sum of all the physiological states an organism passes through from the egg or young to the adult. This in itself recognizes the fact that an animal is a complex of physiological states, and that these states vary during the life process of the animal. Among the various points which might be emphasized in connection with physiological life cycles one interests us particularly, namely, that the physiological changes are *rhythmic* in nature—that there is a fixed sequence of physiological states beginning with the breeding adult, passing through the egg, young and old larva, pupa and adult stages (as in the case of the Tiger beetles worked out by Dr. V. E. Shelford) and finally the sexually mature adult. Dr. Shelford has shown that this sexually mature adult returns to a physiological state essentially like that of the young and that they are physiologically equal. The female selects the place for oviposition, and at this time she is in a state of physiological equilibrium with the environment selected. The young hatches in this environment and is likewise in physiological equilibrium with that environment; therefore the female, when laying her eggs, has become physiologically a larva. Dr. Shelford has shown that moist sand is preferred for the laying of the eggs. If this sand becomes too wet the larva will burrow and wander until a new place is found under conditions that are similar to those chosen by the female when laying the eggs. These congenial conditions being found, wandering ceases. Hence the female and the larvae are physiologically alike, and there is no instinct involved in the selection of the breeding grounds; it is a matter of physiological balance. This point is emphasized as I hope to show that this idea can be applied to certain phases of bird migration. For example: It has been demonstrated that the young of some species of birds (robin) show upon leaving the nest a tendency to move northward. This is often held up as a strong evidence to prove that migration is an *inherited instinct.* However, if we admit that the young at the time of immaturity are in the same physiological state as the female at the time of laying the egg, then, if the female was at that time under physiological urge (which we will discuss in a moment) that accompanied a northward migration, is it surprising to find the young temporarily responding to a like physiological state? I think not. Thus this northward migration becomes a response to a physiological state, not a reaction to inherited instinct.

It is well at this point to investigate for a moment what is going on, physiologically, in the reproductive cycle of the higher vertebrates. As the female of the human species approaches the period of sexual maturity, we find marked physical changes occurring throughout the body. These changes are internal as well as external, mental as well as physical. The internal metabolism is markedly increased, fat is deposited, bone deposition increases, the composition of the blood changes in regard to erythrocyte and leucocyte content and, when the period of menstruation occurs (the proöestrum) we find the female a totally different organism from a physiological point of view. The female, at the time of ovulation, has reached the climax of a series of physiological states through which she has been passing. If fertilization occurs during the oestral period, then a new series of physiological states is initiated, through which the female passes, slowly returning to normal only after the birth of the young. If fertilization does not take place, the return to normalcy is much more rapid. In either case, however, it is a *periodic* and *rhythmic* cycle, a fixed sequence of physiological states.

In the case of males of animals that are not permanently in a state of sexual maturity we find a corresponding series of changes, reaching a climax at the time of maturity of the spermatozoa, and declining again to normal after the breeding season. In the case of the male, as the climax state is approached, one finds the development of the secondary sexual characters, and the sexually functional male is essentially a different animal physically and physiologically from the immature or non-breeding male. This change is expressed not only in the physical make-up of the animal but also in its activity. The sexually mature female likewise expresses her change in physiological state by a change in behavior, as in the case of the salmon, the dog, the birds. Thus we can see that the sexually mature animals are different from the non-functional animals not only in physical appearance in many cases, but also in behavior, activity, mental condition—all of which is simply saying that they are in a different physiological state.

Do we know what brings about these changes? We believe we do. Almost endless experiments have shown that these so-called secondary sexual characters and the changes that result during the season of "heat" or "rut" or "desire" are due to the activity of the sex glands. The clasping reaction of the male frog, which results in amplexus, occurs only at the height of the breeding activity, and Steinach (1910) shows that if the testes are removed the clasp reflex is abolished. The effects of castration of both sexes are too well known to demand listing. Thus the sex glands become responsible not only for the development of secondary sex characters, but also for the *behavior* of the animal during the period of activity of the gonads. For the purpose of our discussion it might be well to class behavior under gonad influence as a secondary sex character, which it surely is. Is not migration, then, being a behavior expression of physiological change accompanying gonad activity, from one point of view at least a secondary sex character? Is it not closely associated with the development of secondary sex characters in migratory forms—as the morphological changes in the salmon and the plumage and song alterations among the birds? Since secondary sexual characters, which are ordinarily considered as being *physical* changes in the animal, are admittedly stimulated or repressed by gonad activity or inactivity, is it not conceivable that migration, a *behavior* change at the same time, is likewise an expression of gonad activity?

This brings us inevitably to the question of how the gonads bring about the changes so evidently traced to them. It is a well-known fact that the gonads produce both an *external* and an *internal* secretion. The external secretion is the *gamete*, the internal secretion a *hormone*.

Many proofs of this hormone secretion as the causative factor in the production of secondary sex characters exist. Castration, if carried out before puberty, prevents entirely the development of these characters. Periodic menstruation is indicative of a normal ovary; if the ovary be removed, the phenomenon no longer occurs, but will begin again if ovarian transplantation occurs.

In the gonad itself two types of cells are responsible for the two types of secretions, and it is the *interstitial* cells which are the hormone source. These cells lie entirely outside of the tubes that produce the external secretion and are quite definitely separated from them. It is possible in the male to tie off the tube containing the sex elements, thus causing them to disappear entirely. What is the result? The interstitial cells remain, and the point is this: that in such a case the sex instincts remain as strong as ever and the secondary sex characters develop normally. If the testes of a buck are exposed to X-rays the generative cells are killed, but not the interstitial cells. The antlers of the buck undergo no change whatever, while if the buck were castrated—in which case the generative cells as well as the interstitial cells would be lacking—the antlers would be lost. Thus we see the effect of the hormone secretion of the interstitial glands. The work of Steinach, Brown-Sequard, Voronoff and others points clearly to this. That the blood stream is the carrier of substances indispensable to reproduction was shown by Von der Heide, who injected foetal blood serum into a pregnant woman and evoked labor pains. The source of this hormone in the female has been shown to be the corpus luteum. L. Loeb (1911) has shown that the corpus luteum prepares a hormone which sensitizes the uterine mucosa so that it reacts to the stimulation of the ovum, with the production of the maternal placenta. He has also shown that this sex hormone produces the changes in the breast as well as the fact that menstruation is due to an influence emanating from the ovary during ovulation and, reaching the uterus, causes a severe congestion which produces menstruation. That similar changes, due to similar causes, are going on in other higher vertebrates can not be doubted, though in a vast majority of cases no demonstration has been attempted. We still know practically nothing of the physiological changes in birds, but a supposition that assumes a similarity of circumstances is not out of place since we know something of hormone behavior.

Let us look at some other vertebrates and see how things stand. We know that in the case of the dog each breed has its own breeding cycle and that individual females possess their own individual cycle to which they remain very constant, irregularities occurring most noticeably with advancing age. We know that there are profound changes going on internally in the female as the breeding period approaches. The proöestrum is evidenced externally by the swollen vulva and the discharge of bloody mucus. This discharge ceases with the beginning of the oestrus, or period of desire, which lasts from seven to ten days. During this time the female is a very different animal: ordinarily quiet, she now takes to roaming and is incessantly on the go. Dog breeders tell us she is looking for a mate, but is this activity not merely a response to hormone secretion? Have not the hormones produced during the processes going on internally thrown her out of physiological equilibrium with her environment for the time being? Remove her ovaries so that the corpus luteum can not form after ovulation, and she does not roam. Are these roamings not periodic? Then they come under the definition of migration: they are a type of migration. Take the case of the frog. In the spring the males precede the females to the pond, and we find that they are sexually mature first. Does not this in itself indicate a sex complex? The same is true of many species of fish. In the spring the pickerel migrate into the very shallow water of the marsh overflow. The male is mature first and may precede the female. In my work on the cisco I find the males preceding the females in the breeding migration by several days, and that they are sexually mature first. In the case of birds the males of some species migrate ahead of the females—the red-winged blackbird and penguin are cases in point. Non-breeding birds often do not migrate nearly as far as do the breeders: the herring gulls that remain far south of the breeding grounds are non-breeders. Do these facts not indicate a sex complex in connection with migration? The singing of the male bird is characteristic of the spring migration and the period following. Why does he sing? Is he happy? Probably he is no happier than he will be a little later in the season when he is silent. His metabolic processes are speeded up by the activity of his reproductive hormones and the result finds expression in song as in other species it may find expression in color or plumage modifications or odd actions, as the dance of the prairie chicken and the drumming of the grouse. We know these phenomena as secondary sexual characters and it would seem that they are due to hormone secretion. Yet, among our song birds at least, they occur only in connection with the migration. Does this mean nothing? It is far too general and far too common to be of no significance.

Another point. The big waves of bird migration occur almost at the exact date year after year, irrespective of the weather *to a very large extent.* If external factors alone were responsible for migration the birds would arrive almost any time. But an organism among the higher forms has a definite cycle through which it passes —a very definite series of physiological states which are passed through in sequence and which are not materially hurried or retarded by slight changes in external conditions. Therefore it is not surprising to find the migration waves occurring year after year with surprising regularity, and to find that year after year the same species are found in the same migration waves. Species having similar breeding cycles find themselves migrating at the same time, just as they find themselves developing bright colors or singing or dancing at the same time. To me these are all expressions of one and the same internal cause: hormone secretion.

As we have thus far based our discussion on the idea of internal change due to a hormone cause, the question naturally arises: is this pure speculation? Is there any ground at all to support such an idea? I believe there is, and would call attention to the interesting case among cattle, known as the "freemartin." The freemartin is a sterile individual cotwin to a normal bull calf. The work of Lillie has shown that this sterile individual is a sexually undeveloped *female* in spite of the fact that it shows unmistakable secondary sexual characteristics of the *male*. Embryologically the freemartin and its normal male twin are dizygotic, but monorchordial. There is established between the twins a direct blood flow, so that the arteries of the twins are in direct connection. Thus the blood of one foetus circulates freely in the body of its twin. Now, the male is throwing off hormones into the blood and these, carried into the body of its female twin, repress the normal development of her reproductive system and at the same time stimulate the development of certain secondary male characters. Thus we find a profound internal change due to a hormone cause: not only a nullification of sex, but a development of secondary characters of the *opposite* sex! These facts show further either that (1) the male hormone is stronger than that of the female or (2) that it is developed earlier and so gets a head start in its activities. Either of these ideas might well explain why in some migratory species of birds one sex (the male) always precedes the other in the northward movement. Another case: The obstetrician knows of cases where newly born girl babies menstruate shortly after birth. The explanation lies in the fact that the child has absorbed from the mother certain hormones which, had the mother not been pregnant, would have caused her to menstruate. A contribution by Poehl, Zoeth and Pregel may prove to be of great importance. These men have shown that "spermin," the hormone produced by the testes, lessens fatigue and causes an increase in the amount of muscular work that an individual may accomplish. This is indeed very significant when one considers the astonishing distances covered during migrations and the tremendous expenditure of physical exertion involved.

If these things are true, then what are we to say about the non-migratory species? Simply this: that their tolerance is greater than in the case of migratory species. Barring physical barriers, an organism is limited primarily by the *range* of its regulatory mechanisms, as we have already pointed out, and by the *degree* of its adaptability. Non-migratory species are such because the degree of physiological change due to the activities of the gonads is insufficient to throw them out of harmony with the environment: their range of tolerance is greater. It is a noteworthy fact among our birds that do not migrate that there is relatively little development of marked secondary sexual characters, as in the English sparrow, the downy and hairy woodpeckers, the crow—while a vast majority of migrants show decided development of either plumage or voice. Furthermore, that migration is not a response to external conditions is hinted at by the fact that when a non-migratory species is transplanted from one continent to another, it remains a non-migrant, as in the case of the English sparrow and the starling.

Again, I believe that specific differences are more than a matter of color of a feather or number of primaries or length of toe. I believe that species are physiologically and perhaps cytologically different. With this idea in mind, I do not find it surprising, therefore, to find the striking differences between closely related species. Close gross structural relationship does not necessarily imply similarity in physiological states. Among the plover, for example, it is not surprising to find the far-reaching migration of the golden plover and the relatively limited migration of the killdeer. It is simply a matter of degree of change of physiological states, coupled with a varying range of tolerance. Other things being equal, the greater the change the greater the migration.

There is yet another phase that, while not directly in line with a discussion of migration, is in line with a consideration of physiological state and change which we are discussing, a phase which I believe has for the most part been overlooked. It has already been pointed out that the breeding female is in an essentially different physiological state from the non-breeder. The most marked change is a change in her behavior as expressed in terms of nest-building and incubation, two closely related phenomena which are conspicuous in the non-breeder by their absence. Therefore, they form a part of the breeding cycle and an external change due to an internal hormone cause. These phenomena are ordinarily held up as examples of parental instinct, maternal affection, intelligence and what not. Let us examine them for a moment. In the petrel we have a bird that spends its entire life over the water, flying over the waves of the oceans for months on end. At the breeding season the birds come to land, build their nests and incubate, a most radical departure from their normal mode of life. The same is true of the albatross, the gulls, the terns. The breeding bird sitting on her eggs is, physiologically, a different bird. What is this difference? Expressed in ecological terms, she has become "thigmotactic." The approach of this thigmotaxis is first seen in the nest-building: the moulding of the nest to the body. Here is your petrel or gull or albatross or robin suddenly seeking the thing which all its life it has avoided—general contact with surfaces. The bird gouges out a depression in the sand or constructs a nest, moulding the contour to the curvature of the breast. The thigmotactic response reaches its culmination in the three or four weeks of patient incubation: how different from the normal behavior of the bird! This thigmotaxis wears off again in the course of time, particularly evident if the eggs do not hatch: the bird does not sit indefinitely. In the case of the woodcock, so strong is this impulse that it is possible to walk up to an incubating bird and lift it from the nest by hand and eventually replace it. This is often paraded under the title of "protective coloration"—that the bird trusts to its resemblance to its environment for protection. While I believe in the existence of protective coloration, I do not believe that the woodcock has such unbounded faith in the theory as to permit itself to be handled. On the contrary, I believe that the thigmotactic response is simply too much for it to overcome—stronger even than fear. From this point of view, then, nest-building and incubation become thigmotactic phenomena accompanying the culmination of the physiological changes due to the reproductive cycle, and are, like migration, expressions of a hormone cause.

An animal in nature is in a state of stable equilibrium with its environment. Any change on the part of the environment must be met by a corresponding change on the part of the organism either by (1) an adaptive reaction to enable the organism to meet this change; or (2) by a retreat of the organism. "The disharmonic organism must attune itself or perish." Since, therefore, an environmental change produces a reaction on the part of the organism due to the upsetting of the equilibrium which must exist between that organism and its environment, it is reasonable, as we have shown, to suppose that a change on the part of the organism which throws it out of physiological equilibrium with its environment is capable of causing the organism to move into a new environment. Since an animal in the breeding season is physiologically a very different animal from the same organism in the non-breeding season, and since the change is due primarily to the activity of the gonads, it follows that the movement of the organism which causes it to seek a new environment (in which it becomes at least temporarily a balanced unit) is due directly to the activity of the gonads. Thus migration becomes an adaptive response to gonactivity, and since this activity is a hormone secretion, migration becomes an *effect* to a hormone *cause.*

This idea of migration as an external expression of an internal urge takes all idea of abstract instinct out of the problem. It does more than that: it gives us a concrete basis for explaining the changes that accompany the appearance of the breeding season and a basis that is subject eventually to proof or disproof by scientific procedure. Just how the hormone of the sex glands brings about these changes can not as yet be stated: our knowledge of hormone activity is as yet very inadequate. So we come here to a locked door. But there is a light under the door which shows that there is something on the other side, and it is confidently believed that the key to the door is not lost, merely not yet found. Perhaps there is a change in the hydrogen ion concentration of the body: there is a hint of this in the only published work done on hydrogen ion concentration of pregnancy, by Löb and Higuchi (1910), where the investigators found an ion concentration of the normal placenta of pH 7.65 and that of the foetal placenta pH 7.96. In closing, a word to those who hold that migration is due to wind or temperature or pressure or humidity or length of day: hormone activity changes the entire physical status of the organism and thereby may, perhaps, form a basis for the organism's reaction to wind or temperature or what not, by having upset the internal equilibrium and by increasing thereby the organism's susceptibility to external change.

DINOSAUR EXTINCTION

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THE riddle of dinosaur disappearance excites wonder. That stupendous group lived through all Mesozoic time, showed considerable plasticity, reached armor specializations paralleling those which seem to give the Testudinata an exceedingly long lease of life and finally increased its numbers in relatively modern environments. Yet the higher or more bird-like types, the sauropods, the carnivores and many armored forms all alike vanished almost instantaneously as geologic time goes. Such an obliteration seems strange in a world "which makes its own climates."

A searching analysis of the fine fossil vegetation of the English lower Greensand reveals a barely subtropic climate, though some dinosaurs were there present. Equable and varied habitats were always left over to the dinosaurs, as their range through the higher latitudes was in any degree restricted or cut off. Nothing clearly known of food habits or of early Tertiary climates explains that abrupt ending. "There was little about the dinosaurs prophetic of their fate." What then were some of the invisible influences that undid them? Were the greater enemies those within or those without the phylum?

Thirty years before the discovery of the Manchurian eggs, Forsyth-Major, assuming that reproduction was by means of eggs, averred that the sauropods especially were brought to extinction by raids of early mammals on the eggs. The view is also more or less correctly attributed to Cope. But the hard tough eggshells of many of the dinosaurs could not readily have been broken by most smaller animals, and the behavior of the varanid lizards shows that all really formidable egg-eaters unite with great activity in the habit of swallowing eggs whole. The potent feeders on dinosaur eggs and young must be sought for amongst the dinosaurians themselves—and perchance, amongst the earliest varanids and boids, both of which reached immense size by Eocene time. Any enemies within the early Creodont lines are altogether hidden in the background.

Struthiomimus, the "ostrich mimic," and *Ornitholestes,* the "bird robber," have had the attention of paleobiologists. These were the weaker carnivores, the kind becoming toothless towards the close of dinosaur times. *Laelaps* and the other large carnivores have been freely pictured in combat with the sauropods or fighting amongst themselves, and the view is not unreal. I well remember Cope's classroom remark that "Laelaps was one of the most aggressive and formidable animals that ever existed." But the idea presented here is a somewhat different one of incessant egg robbery, of continual prowling throughout the egg season, and then the hunting down of the young.

A further item in the carnivore *menu,* serving when other food grew scarce, and also suggested from the habits of *Varanus* is easily understood. Just as I found with the skeleton of the great Pierre turtle *Archelon* a single tooth of a shark, identified by Cope as a broad-toothed *Lamna,* so I found a single weakly serrate, rather flat carnivore tooth (Allosaurus?) with the vertebræ of the giraffe-necked sauropod *Barosaurus.* In both instances scavengers are probably indicated.

The plant-eating dinosaurs were numerous. They had an immense range in space and time, and the point is suggested that their eggs, eaten during a season of some length, were one of the stimuli to increase of size in the carnivores; that these after a time became very destructive, and (somewhat overgrown) were in turn subject to the loss of eggs and young due to the depredations of animals less powerful than themselves. It was in the midst of such life that mammalian viviparation became, partly of outer necessity, a fixed mode of reproduction.

Neither size, number or nutritive value of reptile eggs now or in the past is in doubt. In the markets of Burmah, Varanus eggs are esteemed and sold at a higher price than hen's eggs. Free from all disagreeable taint, of finest flavor, the white does not, as in fowls' eggs, coagulate on boiling; and these same fine qualities I have found in eating the eggs of the wood tortoise. The flesh of reptiles may have a protective rankness or even a poisonous or injurious quality, as in some of the turtles, and the eggs yet be wholesome.

Tyrannosaurus, amongst all carnivorous dinosaurs, most suggests a growth and habit due to some increased abundance of prey. What more likely than that the immediate ancestors of this dinosaur got their first impulse toward gigantism on a diet of sauropod eggs, and that, aside from the varanids, the theropod dinosaurs were the great egg-eaters of all time? The idea does not preclude a final increase in size passing the limits of food supply and so tending toward extinction. Very great sizes in organs or individuals may pass the vantage point through inertia; and evidently the dinosaurians so suffered.

That the earlier varanids and boïds also aided in the final destruction of the dinosaurs would be quite inferable, were the skeletal remains of all found associated in the fossil quarries, and that they are not so found may be due to accident or habitat. Just as the dinosaur record closes, the great Eocene varanid of crocodilian size from Wyoming described by Gilmore and also the boas (*Paleophus, Boavus, Dinylisia* of the older Eocene) appear. Such giants had ancestors somewhere in late dinosaur times. Both varanids and boids retain great size throughout Tertiary time, and both seem to have passed the acme of their development but recently. The varanid *Miolania prisca* Owen, of the Queensland Pleistocene, was a startling reptilian thirty-three feet in length.

Falling far short of such proportions, the varanids of to-day are nevertheless formidable marauders. They are seen all over the Old World tropics from desert to jungle, preying on the largest animals they can master. They climb trees, are expert swimmers, and, though hiding their own eggs in hollow trees, are everywhere great prowlers and egg-eaters. Their traits and habits afford a veritable foreground to the portrayal of carnivorous dinosaurian life—dissimilar, mainly, as indicated by pneumatic and other birdlike skeletal features, and a prevailing bipedality. *Varanus salvator,* the great monitor of Ceylon, or "kaboragoya" is the largest living lizard, having a length of eight feet. As Ditmars significantly relates:

> This powerful brute is partial to the jungles. Few sportsmen have been through such a country without occasionally being startled by the rush of a monitor ahead, making as much noise as some hoofed animal as it tears its way through the undergrowth. The methods of attacking a small animal resemble more the actions of a warm-blooded animal than a reptile. Rushing for the ill-fated creature at a gait rapidly overtaking it, the lizard seizes the prey and shakes it in the same violent fashion as a terrier shakes a rat. If the animal's struggles become so violent that its escape is rendered possible, the reptile holds it to the ground under its strong claws—as long as those of a fair-sized leopard, while the jaws take a better hold. The animal is then swallowed whole.

A caged example had a great appetite for eggs, as Ditmars continues:

> Like all monitors, this one is fond of eggs, taking eight to ten hen's eggs at a meal, swallowing them entire and with such rapidity that the eggs come in contact with each other in passing down the throat, producing a clicking sound that can be heard plainly by one standing nearby. Digestion is rapid. Within twenty-four hours the gastric juices have thoroughly disintegrated the shells which are broken by compression of the stomach. Lizards and snakes are also eaten by this monitor.

Turning again to possible dinosaur habits the view presented is that the tooth-set gape of *Tyrannosaurus* is not inconsistent with nurture on sauropod eggs. Limning in the picture, it is *unlikely* that the dinosaurs merely dug in their eggs (certainly not the more birdlike forms), and it is *likely* that some care was given to the eggs and the young. Certain frill lacerations in Ceratopsians are held by Lull as possibly due to conflicts among the bulls, and such contests in higher animals at least indicate copulatory interest and go with maternal care. Probably, too, the dinosaurian group being such a varied one, the carnivores were the more active blooded kind, whence the sauropod females may be imagined guarding their eggs as does one of the Boas. While as the days passed and the hatch shifted along the belly or the coiled tail ever ready to strike, the alert Tyrannosaurus gathered up any scattered eggs. Or the eggs if left unprotected would be the more quickly raided. The young, too, must have been subject to raiding and "cutting out," as in mammals. Perhaps the carnivores at times became social, hunting eggs and other booty in droves. With such active and powerful beasts at the jungle-edge, life was varied and sanguinary, be it within scientific dignity to say so.

How the dinosaurs lived and produced their young becomes less obscure as the facts of distribution and association are assembled. Thus the frequency with which dinosaurians of varied type are found in marine beds leads to a suspicion that there was a certain degree of seasonal dependence on water habitats. Bayou protection in winter has already been suggested. How fossil occurrence might fail to disclose the essential or average habitat and life associations can be illustrated in this way. The great "leatherback" turtle often wanders out of the warmer waters of the Gulf Stream, becoming helpless within North Atlantic shelf areas of fluviatile deposition favorable to fossilization. In such instances the resulting fossils, if brought to light in some future age after the course of the ancient currents could no longer be fixed, would yield one-sided or even misleading environmental evidence. The lure that entices the leatherback so far from the equable limits must be the northern animal forage, accessible only by way of the warm currents. The true habitat of any vertebrate might well be defined as only the region within which the young are safely brought forth.

To those inferential ideas of reptilian traits and growth just outlined there is this one lesser but not unimportant limit to set. It is possible that the late Cretaceous and early Eocene record must be viewed virtually as it now stands. In that case the Varanid-Boid gigantism was not coeval with the last of the dinosauria, and merely followed early increase in mammalian prey; although the argument remains much the same. If the herbivore reptilians evolved or grew up on a changing pasturage, and that would only be following life's simplest way and mean, there comes into view the correspondent spur toward carnivore growth—more varied prey. Both stocks reacted to the nearest available food sources as they underwent the biotic cycle.

In the larger view, the experiments of zoologists show the profound effect of food-change on reproduction and growth; geneticists find progressive change a thing outside their observation, time thus being the first essential in the origin of new species; and the paleontologist sees plainly enough that phyletic development, once begun, inclines to rapidity, reaches a climax and may then slow down after a certain amount of senile variety. Extinction of the phylum pursues much the same course as its obverse, development; after the more general disappearance, a few isolated forms are usually left behind long ages after all the rest of their kind are gone. Taking an example from the invertebrate world, the rise and going out of the trilobite is thus portrayed by Walcott:

> The trilobite slowly developed in pre-Cambrian time, reached its maximum in the Cambrian period, and continued on in full tide until well into Ordovician time when the sea bottoms became crowded with a large and varied fauna, and numerous enemies, some small and insidious, parasitic in nature, others large and powerful, appeared, together with various types which, while not physically antagonistic, were economically so in being better adapted to live in the same manner under the same conditions. It kept up the struggle, but already an ancient type, it had lost its juvenile race plasticity, and ability to modify itself to meet the new conditions. Never having penetrated into fresh or other non-marine waters, or into the deep sea, those havens of refuge where the relics of many ancient types may still be found, the trilobite, unable to cope with the new world in which it found itself, was consumed as food by its new enemies, both internal and external, and at the same time subjected to overwhelming competition, so that the individuals died off more rapidly than they could reproduce, and the race disappeared with the close of Paleozoic time. It persisted for many million years and left its remains more or less abundantly through about 75,000 feet of stratified rocks.

Briefly told, the dinosaurs lasted through as great a deposition of sedimentaries as the trilobites; though in duration they are far exceeded by the mammals which have so recently passed their zenith. Beyond any other race the dinosaur, after the great deployment, half way through his history, goes out with a mighty flash of variety; eight or more remarkable genera are already known from the American Lance (and Ojo Alamo) alone. But neither the food supply nor geologic change affords a direct explanation for such a sudden termination of all the greater divisions of the race. Nor does early mammal egg-eating do so. About all that can be said is that the greatest enemies of the dinosaurs seem to have been of their own kind, and that perchance early varanid egg-eating and both varanid and ophidian attacks on the young brought them nearer the end, if any survived into the Eocene.

So stands the riddle. Was there some more recondite factor which forced the dinosauria out of the world's vertebrate life? Especially Abel, following somewhat Huxley and Marsh, sees much to emphasize in the bird-like features indicating early arboricolous, or tree-dwelling habits. The types on the borderland between the birds and the more terrestrial dinosaurs are expressively described by Abel as the Avidinosauria; and no one disputes the direct affinity implied. Nor can the avian resemblances in the skeleton of the dinosaurs lack a deep physiological significance in either their origin or their later expression. Other opinion *per contra,* that is why the dinosaurs were as a group warmer blooded relatively to the climates of their day than lizards and crocodiles are now, though probably not so warm-blooded as the early birds. I say taken as a group, because I do not suppose that the widely different families all had the same blood temperature. It is recalled that Baur, a keen anatomist, even argued that the Dinosauria were too varied to constitute a natural group. Any other temperature conception would amount to virtual denial of the potency of the geologic environment to produce warm-bloodedness at all. Need any one be reminded that the great transition from very ancient life into mammals; birds and dinosaurs was simultaneous, and coordinated with the changes that led to the flowering plants? Whatever the primal impulse, reaction to it was at its peak in the late Permian and early Triassic. It involved as well the origin of the pterodactyls.

In truth, warm-bloodedness can only be a response to persistent and progressive factors common to all terrestrial environments. And certainly the most potential of such factors within any fairly uniformitarian purview of climate and the general course of cosmic change would be variation in respiratory nitrogen, oxygen and carbon dioxide, as mainly controlled by the oxygen release in the photosynthetic processes of more and more dicotyledonous forests. As expressed by Vernadsky, quoted by Nichols:

> We know of not a single reaction in the earth crust which would liberate free oxygen except its production by organisms containing chlorophyll in the synthesis of their organic substances. At the same time we know of thousands of chemical reactions in the earth crust [and soil cover] which consume oxygen. If there were no chlorophyll bearing organisms, free oxygen in the atmosphere would long ago have ceased to exist.

Variable amounts of atmospheric carbon dioxide and some definite variation in the rate at which oxygen has been thrown off by successive floras are, then, a certainty. It can not be believed that the early Triassic forests of ferns, equisetes, seed ferns, cycads, cycadeoids, varied conifers, cordaites, ginkgos and pre-dicots and monocots set oxygen free at the same rate as the later Mesozoic forests. Moreover, it is likely that the withdrawal of carbon dioxide in the Pennsylvanian limestone-making and of carbon in the laying down of the coal were early causes of Permian glaciation; while another very great disturbance of the atmospheric equilibrium is indicated by the thick Cretaceous limestones. Generally speaking, the impression gained is that carbon dioxide, the breathing stimulant, mainly supplied from inorganic sources, grew less toward Tertiary time, and that although the areas of ozone-laden air lessened as the greater conifer forests receded, respiratory oxygen tended to increase. And herein must have lain the elemental need and possibility of adjustment in the warm-blooded vertebrate life of the upper Mesozoic.

Nevertheless it was there, after having at first so sharply responded to the influences that brought about feathered and haired life, that the dinosaur lagged. The verge of progress was reached but never passed. It was left for other races to acquire flight, and at last even the science of mathematics! The organs of respiration were not very different from those of the birds in which the respiratory function reached intensity; the early reptilian stage was passed; the mammals were acquiring the smallest and most numerous of blood disks, so that in them the effective surface of organic interchange reached a maximum. The necessity for progressive physiologic change became tense as Cretaceous time waned; but the response, so far as inherently possible (or not inhibited by aplasticity), first came too slowly, and then overleaped the bounds. To a world that ever went forward, the final dinosaurian reflex manifested itself in absurd or fantastic armor patterns, little else than experiments in resistance to change.

At all points the dinosaurs that had trampled the earth till the grasses grew, the most superb of all vertebrates, the creatures that fix the imagination above all others, are seen to fail. The growth forces and the responses to environment were no longer in adjustment. Eggs were few, their loss from attack devastating, life slow. The young were the prey of their own kind, and the race had lived long enough for reptiles lower in life's scale to threaten. If new enemies were needed they were at the jungle-edge. Geologic change that once would have meant mere fluctuation in habitat affected the declining numbers disastrously, and what such change and the reptiles soon to rule in the forests of the Eocene may have failed to accomplish, senility did. That long dinosaurian day was done. Its sun was sinking beneath the horizon forever.

SHORTER ARTICLES AND DISCUSSION

SIZE OF COB IN MAIZE AS AFFECTED BY THE NUMBER OF FERTILIZED OVULES

SOME measure of yield often is desired in progenies of maize having a number of hand pollinated ears. Since hand pollinated ears frequently are fertilized imperfectly the actual weight of seed produced by them is not a fair measure of their yield.

For a number of years past we have made use of cob length and number of rows as the best expression of yielding capacity within or between closely related strains but recently evidence has been found in some progenies that the length of the cob increases in proportion to the number of fertilized ovules to an extent which if found to be general would vitiate yield comparisons based on the length of the ear.

This evidence appeared in data accumulated to study the factors influencing variability in the amount of crossingover in a hybrid between Chinese waxy and an Algerian pop corn. During the progress of this investigation we had occasion to measure the relative effects of single and double applications of pollen on a number of ear characters.

Quite unexpectedly these measurements showed that in addition to an increased number of seeds the ears resulting from two applications of pollen were longer and had more rows than those resulting from a single pollination. The biometrical constants for this population are shown in the following table.

From this table it is evident that ears having two pollinations produce more seeds than those having but a

Character of Pollination	Cob length (cm.)	Number rows	Base of ear without seeds (cm.)	Tip of ear without seed (cm.)	Number of seeds	Date of[1] pollination
Two pollinations	18.44 ± .56	18.78 ± .38	.167 ± .19	1.00 ± .21	586 ± 30.0	13.94 ± .62
One pollination	15.90 ± .24	17.44 ± .16	.766 ± .08	1.22 ± .09	417 ± 13	18.45 ± .27
Difference	2.54 ± .61	1.34 ± .41	.599 ± .20	.22 ± .23	169 ± 32.0	4.51 ± .67

single application of pollen. Apparently this increase in number of seeds is accomplished by an increase in cob length, a reduction in naked base and an increase in number of rows, but curiously enough there is no clear reduction in the naked tip which for these ears appears to be a constant.

That the observed differences in length of cob and in number of rows can be attributed definitely to the number of pollinations is not so clear since there obviously is a significant difference in the average date on which double and single pollinations were made. This difference is entirely accidental, the second application of pollen being made according to the general appearance of the silks, but the observed relationship of single and double pollinations to cob length and row number may be simply a secondary one due to a possible correlation of earliness with these characters. To determine this point the correlations between these several characters may be examined.

COEFFICIENTS OF CORRELATION

	No. of pollinations	Cob length	No. of rows	Date of pollination
No. of pollinations[2]		.403	.312	-.637
Cob length	.403		-.126	-.239
Number rows	.312	-.126		-.010
Date of pollination	-.637	-.239	-.010	

The table shows a significant negative correlation between date of pollination and cob length, indicating that in this progeny early plants have the longer ears. If the partial correlation is calculated for number of pollinations and cob length for constant date of pollination it is found that the coefficient is reduced from .403 to .335 ± .062, a coefficient still too large to be ascribed to chance. The partial correlation between number of rows and number of pollinations for constant date of pollination is found to be .395 ± .059, an increase from .312. Apparently the increase in ear size coincident with more than one application of pollen can not be considered as the indirect result of the correlation of ear size with earliness and the explanation must be sought elsewhere.

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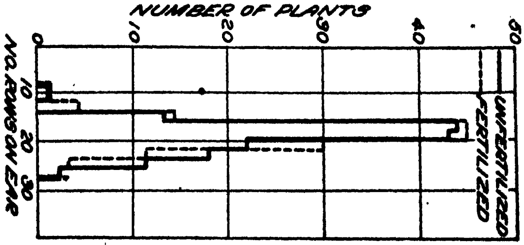
[1] Number of days taken from August 1.

[2] Biserial coefficients calculated for this character.

It seems not unlikely that in making second pollinations there is an unconscious selection toward large ears, since such ears would be likely to produce a larger number of silks following the initial pollination than would small ears and hence appear in need of additional pollinations. While this explanation of the observed differences would seem entirely reasonable, especially in view of the increase in row number, it was thought best to obtain further data before definitely rejecting the possibility of fertilization having a stimulating effect on the length of the ear.



Frequency distributions for number of rows on fertilized and unfertilized ears.

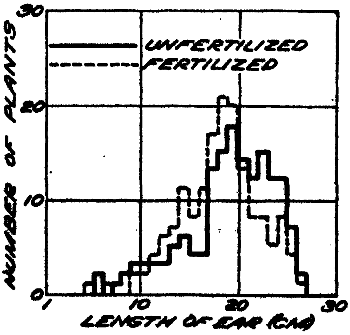
Since the ear of corn at the time of sexual maturity is very small as compared with its mature size, there is a possibility that elongation might be stimulated by fertilization in proportion to the number of ovules fertilized.

If this were true the maximum difference should be found when ears completely fertilized are compared with unfertilized ones. To test this point a number of ears on plants chosen at random were protected throughout the season by means of paper bags, thus preventing pollination, and an equal number of plants were permitted to function normally. The strain chosen for this experiment was a variety of dent corn inbred for four generations. The results for cob length and number of rows are shown in the following table and the frequency distributions for these characters are shown graphically in Figs. 1 and 2.

Character of pollination	Cob length (cm.)	Number of rows
Not pollinated	18.705 ± .2439	18.258 ± .165
Pollinated	17.773 ± .2142	17.747 ± .113
Difference	.932 ± .325	.511 ± .196

It is apparent that these differences are in the opposite direction from those obtained with single and double pollinations. They offer no support to the hypothesis that the length of the cob increases with the perfection of pollination, but on the contrary they indicate that the growth period of the cob is extended to a slight degree in the absence of fertilization.

The difference in ear size observed with single and double pollinations, therefore, must be due to some unmeasured factor which influenced the choice of ears given two applications of pollen in such a manner that large



Frequency distributions for length of fertilized and unfertilized ears.

ears received on the average more pollinations than small ones.

While the increased length of cob on unfertilized ears is not significant in populations of the size used larger populations might establish the significance of this increase in length though this increase almost certainly would be less than one cm for cob length and but slightly over one half row for the number of rows. Such difference would be negligible from the standpoint of estimating yields in most progenies where only a small percentage of the plants are hand pollinated.

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CHROMOSOMAL CHIMERAS IN THE TOMATO

IN a study of cross-sections of root tips from tomato plants grown from cuttings two plants were found in which certain roots were tetraploid either wholly or in part, while others were entirely diploid (24 chromosomes). At first it was thought possible that fusion of cells during the healing process of cuttings might have some causal connection with the production of the tetraploid cells. A similar explanation was advanced by Winkler (1916) for tomatoes which produced tetraploid adventitious buds following grafting with nightshade. While this possibility has not been entirely excluded for our material it is rendered improbable by the finding of a seed plant with a similar mixture of diploid and tetraploid cells in its roots. This plant was remarkable for the "wiry" or extreme "shoestring" character of its leaves which greatly resemble the leaf condition in an extreme case of mosaic. Probably both of the other plants with tetraploid areas were slightly infected with mosaic. It may

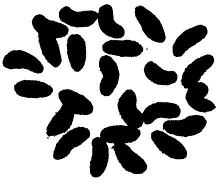

FIG. 1. Diploid chromosome group

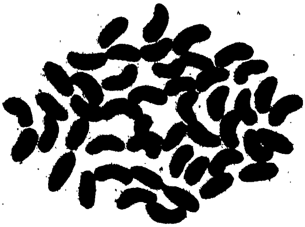

FIG. 2. Tetraploid chromosome group

be possible that local changes due to this disease might affect mitotic processes. The tendency to the production of cells with double, quadruple, etc., numbers of chromosomes in somatic tissues has been considered as an abnormal process related to degeneration as in the digestive epithelium of the mosquito (Holt, 1917) or as a response to certain specific external stimuli. Whether or not the latter can be proven to be the case in the tomato remains to be seen. Certainly the tetraploid areas in tomato roots do not represent degenerative changes. Němec (1903) first reported the production of tetraploid areas in roots by exposing them to narcotics. This work was repeated and extended by Sakamura (1920) who finds that the gall-producing secretions of *Heterodera* produce the same effect. Blakeslee and Belling (1924) found tetraploid shoots on diploid *Datura* plants after treatment with cold. It is improbable that cold is a causal factor in the production of the tomato chimeras since the roots alone appear to be affected at least in any degree. It should be noted that Winkler suggested that tetraploid cells already present might form adventitious buds following wounding. He thinks that this is not the usual cause because he found tetraploid cells very rarely in the shoots of the ungrafted plants which were examined. Thus far the affected plants have shown no tendency to produce gigantic shoots of the type of Winkler's tetraploid tomato. An attempt to obtain plants from root cuttings was a failure.

One of the main points of interest in the frequent occurrence of tetraploid cells in tomato tissues lies in the fact that triploid plants occasionally occur (J. W. Lesley and M. C. Mann, 1925). It is not yet proven that diploid gametes are produced, but if tetraploid cells exist in the shoot of otherwise normal diploid tomatoes as Winkler's evidence indicates, it is evident that such a gamete, if viable, uniting with a haploid one would give rise to a triploid plant. Winkler's tetraploid plant was self-sterile and he does not report attempts to cross it with diploid.

The tomato plants from cuttings were normal diploid in appearance and except for the slight infection with mosaic they were not diseased. Interestingly enough the plants were diploid descendants of a triploid plant which makes the presence in them of tetraploid cells seem rather significant. It would be exceedingly difficult to prove that no tetraploid cells occurred in a plant and for that reason a good deal of preliminary work upon untreated plants would be necessary before one could attempt to attack the problem of causation of the chimerical condition discussed above.[1]

In Winkler's material tetraploid cells were rare, but slight differences from the normal chromosome number were comparatively frequent. The reverse appears to be true of the roots of our chimerical plants.

In the chimerical tomato plants studied the entire root or only a few epidermal cells may be tetraploid. No two roots are exactly alike. In one a sector of tetraploid cells extended from

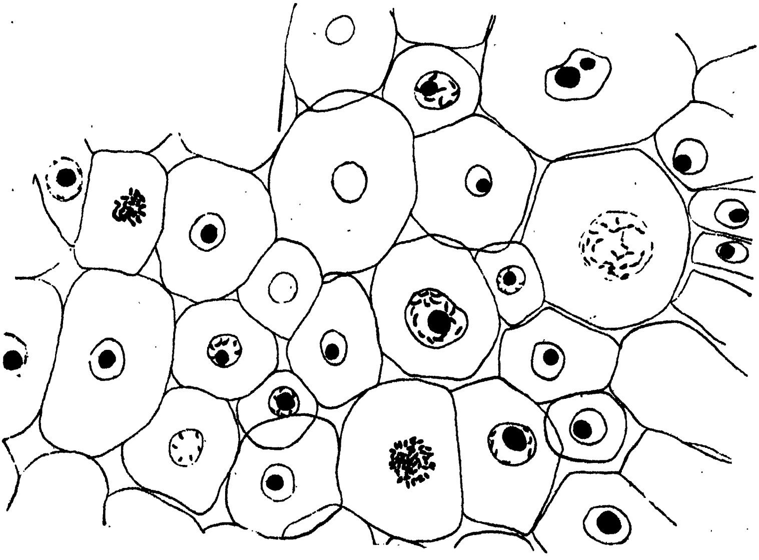

FIG. 3. Tetraploid and diploid regions in the same root

dermatogen to plerome and could be recognized in 77 sections of 8 µ thick or for 616 µ. Since the short first roots were studied the great extent of the tetraploid areas indicate that the chromosomal mutation occurred in the tissue of the shoot. The tetraploid cells are generally larger than the diploid ones, but it will be seen from the cross-section below (Fig. 3) that nuclei which by their size and by the size of the nucleolus which they contain indicate that they have the diploid chromosome number may appear in cells which look quite as large in cross-section as the one containing 48 chromosomes. The approximate areas of the cross-sections of the cells containing metaphase plates were obtained. The area of the diploid cell is 7.04 sq. mm in the original drawing, whereas the tetraploid cell is 13.86 sq. mm, or approximately twice the area at the equator of the spindle. The fact that the roots in the chimeras were not distorted shows that the tetraploid and diploid cells must have multiplied at very nearly the same rate. There is no indication that anything like a reduction division occurs in these roots, and Figs. 1 and 2 show that the chromosomes of the tetraploid are not abnormally shortened as was described for the tetraploid cells of plant roots found following treatment with narcotics, as reported by Némec (1903) and Sakamura (1920), nor do constrictions appear in them.

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[1] In a root of an F_1 between *Crepis biennis* ($N=20$) and *C. foetida* ($N=5$) a few neighboring cells were found having about twice 25 chromosomes, whereas most of the cells contained the expected 25.

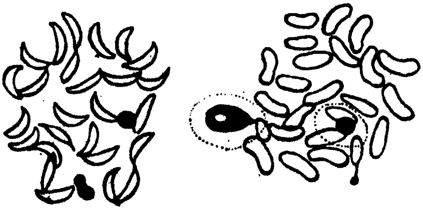

FIG. 4

In the tomato difficulties in chromosome counting often arise from the fact that in root tip material fixed in chrom-acetic-urea the nucleolus gives rise to fragments not very different in size from the very short chromosomes. They are, however, usually slightly out of the plane of the plate and often do not appear at all, *viz.*, in the very late prophase just before the metaphase. The sort of thing which must be taken into account in counting chromosomes of this type is illustrated in Figs. 4 A and B and 5. The chromosomes are outlined, the nucleolar fragments solid black.

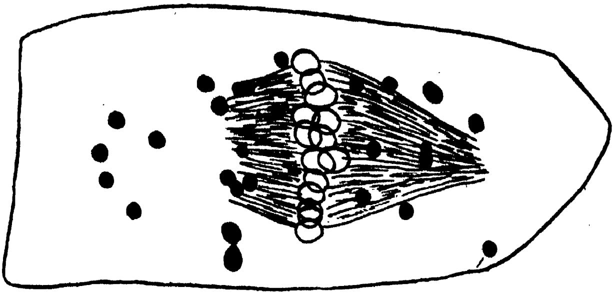

FIG. 5

It is evident that it is advisable that chromosome counts made from a few root tip cells in mitosis be checked by comparison with those of pollen mother cells at least in certain plants.

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